Diploma Thesis

The protein cargo differences of fetoplacental endothelial cell-derived exosomes of women with and without preeclampsia

submitted by

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Graz, am 03.04.2023 *Hanna Weber eh.*

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Abbreviations

ALIX	apoptosis-linked gene 2 interacting protein x
APC	antigen-presenting cells
BMI	body mass index
BMP	bismonoacylglycerolphosphate
CI	confidence interval
DNA	desoxyribonucleic acid
ESCRT	endosomal sorting complex required for transport
EV	extracellular vesicle
EVT	extravillous trophoblast
FasL	FS7-associated cell surface antigen
FCS	fetal calf serum
HBSS	hank's balanced salt solution
HLA	human leukocyte antigen
HPVEC	human placental vascular endothelial cells
HUVEC	human umbilical vein endothelial cells
IFN	interferon
ILV	intraluminal vesicle
miRNA	micro ribonucleic acid
MHC	major histocompatibility complex
MV	microvesicle
MVB	multivesicular body
TNF	tumor necrosis factor
TRAIL	tumor necrosis factor related apoptosis inducing ligand
TSG101	tumor susceptibility gene 101
PBS	phosphate buffered saline
PD-L1	programmed death-ligand 1
PE	preeclampsia
PPARγ	peroxisome-proliferator-activated-receptor gamma
RAB	ras-related in brain
RNA	ribonucleic acid
rpm	rounds per minute

sEng	soluble endoglin
sFlt	soluble FMS-like tyrosine kinase
TBE-T	tris-borate-EDTA + 0.1%Tween®
VEGF	vascular endothelial growth factor
VSMC	vascular smooth muscle cells

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Abstract

Background: Preeclampsia is a severe pregnancy-associated syndrome whose pathomechanisms remain unclear. Since extracellular vesicles, in particular exosomes, are known for their impact on cell-to-cell communication, the question arises whether these vesicles may play key roles in the course of preeclampsia (PE). There is already evidence on the materno-placental side, but little is known about the fetoplacental compartment. We hypothesized differences in the fetoplacental vascular-derived exosomal sorting proteins between normal and PE placentas.

Material and methods: This study analyzed extracellular vesicles from different fetoplacental vascular endothelial cells (n = 21). We isolated and compared endothelial cell-derived exosomes from fetoplacental arteries (n = 7, n = 3) and veins (n = 7, n = 4) from the chorionic plate of normal and PE placentas. All cells were grown under standard conditions. Exosomes were isolated per ultracentrifugation and analyzed regarding the quantity of their surface proteins – specifically ALIX, TSG101, syntenin-1, and CD81 – by immunoblotting. Additionally, we compared the exosomal size distribution and the total amount of protein per exosome, as a calculated variable, in both groups (vessel type and pregnancy course). Statistical analyses were performed in SPSS with Man-Whitney-U tests and a CI of 95%.

Results: The expression of exosomal surface proteins derived from placental veins, compared to those derived from arteries, was similar. Most endosomal trafficking proteins were not expressed in a significantly different manner between PE and normal exosomes namely ALIX ($p = 0.172$), TSG101 ($p = 0.128$), and CD81 ($p = 0.400$). However, syntenin-1 as the most abundant trafficking protein showed decreased expression ($p = 0.031$) in PE exosomes. Size distribution and protein level per exosome did not differ significantly in all groups.

Conclusion: Only syntenin-1, an adaptor protein and mediator for the endosomal sorting complex required for transport (ESCRT), showed a significant decrease in PE vesicles. As this protein affect the cytoskeletal-membrane organization, cell adhesion, protein trafficking and the activation of transcription factors and cell growth, it may contribute to the insufficiency of the placenta in PE. Further research

is required to prove this hypothesis and figure out its exact role in the context of this syndrome.

Zusammenfassung

Hintergrund: Präeklampsie ist eine schwere Schwangerschaftserkrankung, deren Pathomechanismus bisher noch nicht vollends geklärt ist. Da Exosomen für ihre interzelluläre Kommunikation bekannt sind, liegt der Verdacht nahe, dass sie auch in der Präeklampsie eine Schlüsselrolle spielen könnten. Es gibt bereits Evidenz über die Veränderungen von Exosomen auf der maternalen Seite der Plazenta, über die fetoplazentare Seite ist bisher jedoch wenig bekannt.
Wir vermuteten eine Veränderung der Oberflächenproteine, der fetoplazentaren vaskulären Exosomen.
Material und Methoden: Für diese prospektive Analyse wurden verschiedene Proben fetoplazentarer Endothelzellen aus Blutgefäßen der Chorionplatte analysiert (n = 21). Wir untersuchten arterielle (n = 3, n = 7) und venöse (n = 4, n = 7) Proben, von präeklamptischen und von gesunden Plazentas. Die Proben wurden unter Standardbedingungen gezüchtet, die Exosomen wurde mittels Ultrazentrifugation isoliert und im Immunoblot auf die Quantität ihrer Oberflächenproteine ALIX, TSG101, Syntenin-1 und CD81 analysiert. Arterien und Venen wurden separat verglichen. Zusätzlich verglichen wir die Größenverteilung, sowie die Gesamtmenge an Protein pro Exosom, als berechnete Variablen, in beiden Gruppen (Gefäßtyp und Schwangerschaftsverlauf). Die statistischen Analysen wurden in SPSS mittels Man-Whitney-U-Tests und einem Konfidenzintervall von 95% durchgeführt.
Ergebnisse: Beim Vergleich der exosomalen Oberflächenproteine von Venen und Arterien, zeigte keines der Oberflächenproteine einen signifikanten Unterschied. Beim Vergleich derselben Proteine hinsichtlich des Schwangerschaftsverlaufs waren ALIX ($p = 0.172$), TSG101 ($p = 0.128$) und CD81 ($p = 0.400$) nicht signifikant unterschiedlich. Hingegen zeigte Syntenin-1 einen signifikant geringeren Proteinmenge ($p = 0.031$) in den präeklamptischen Proben. Die erhobenen Nebenvariablen (Größenverteilung und Proteinmenge pro Exosom) zeigten keine Signifikanz.
Schlussfolgerung: Von den untersuchten Oberflächenproteinen zeigte nur Syntenin-1, ein Adapterprotein des ESCRT, eine signifikante Abnahme in den präeklamptischen Proben. Syntenin-1 beeinflusst den Aufbau des Zytoskeletts, die

Zelladhäsion, den Proteintransport sowie die Aktivierung von Transkriptions- und Wachstumsfaktoren. Es hat somit möglicherweise auch einen Einfluss auf die Plazentainsuffizienz bei der Präeklampsie. Um dieses Ergebnis zu belegen und den genauen Einfluss auf die Präeklampsie zu eruieren, sind weitere Untersuchungen erforderlich.

1 Introduction

1.1 Exosomes

Intercellular communication is crucial in most physiological processes as well as in diseases. Besides direct and indirect cell contact and interaction with soluble factors, research on extracellular vesicles (EVs), in particular exosomes, has caused increased interest in the last couple of years. It has been shown that these vesicles may contribute to cell-to-cell communication (1). EVs are a heterogeneous group of cell-derived membrane vesicles which are released into the extracellular space. They can be classified into three subtypes based on their origin and function. Apoptotic bodies (size range between 800 and 5000 nm) originate from apoptotic cellular processes and microvesicles (MV), which are formed by budding of the plasma membrane (size range between 100 and 1000 nm). Compared to these, exosomes with a size range between 30 and 150 nm are the smallest type of EV's (2). Exosomes were first recognized as extracellular vesicles in two different studies about reticulocyte maturation in 1983 (3,4) and named after it in 1987 (5). Back then, they were considered cell debris. Nowadays, studies show their impact on intercellular communication. The size of exosomes is heterogeneously distributed and ranges between 30-150 nm. They are found in almost every cell type and many fluids, including urine, sperm, tears, saliva, milk, synovial, bronchoalveolar, amniotic, and vaginal fluids (6). Exosomes carry a wide range of molecules including proteins, bioactive lipids as well as DNA, microRNA, and messenger RNA (7). The function of exosomal cargo is very varied. Exosomes carry MHC II antigen-presenting molecules and take part in immune response (8), presenting tumor peptides and triggering the response (9) or suppressing or promoting viral infection. A better understanding of how exosomes physiologically act could offer new insights into the formation of diseases. The omnipresence of exosomes in fluids could enable liquid biopsies and ultimately allow for early-stage diagnostics. Furthermore, exosomes could be used as pharmaceutical vectors as they are able to enter targeted cells.

1.2 Biogenesis, secretion, and uptake of exosomes

Exosomes arise from the endosomes of cells (Figure 1). This process involves a double invagination of the cells' plasma membrane. First, by endocytosis of extracellular components and the formation of a new cup-shaped early endosome which contains soluble proteins and those from the plasma membrane (10).

The early endosome receives material from the Golgi and performs initial sorting by directing molecules back to the membrane or the trans-Golgi. It eventually matures into the late endosome (11). There, the second invagination gives rise to intraluminal vesicles (ILVs), which are formed by inward budding of the endosomal membrane, thus developing into a mature exosome (12). Besides cell membrane proteins and molecules from the plasma, there are also '*de novo'* proteins from the Golgi complex and the endoplasmic reticulum sorted into the ILVs (13). After this second sorting process, the endosome is called the multivesicular body (MVB). The MVB either fuses with lysosomes and degrades into the plasma or fuses with the plasma membrane and releases exosomes to the extracellular space (14). Many molecules and pathways are involved in the formation of ILVs.

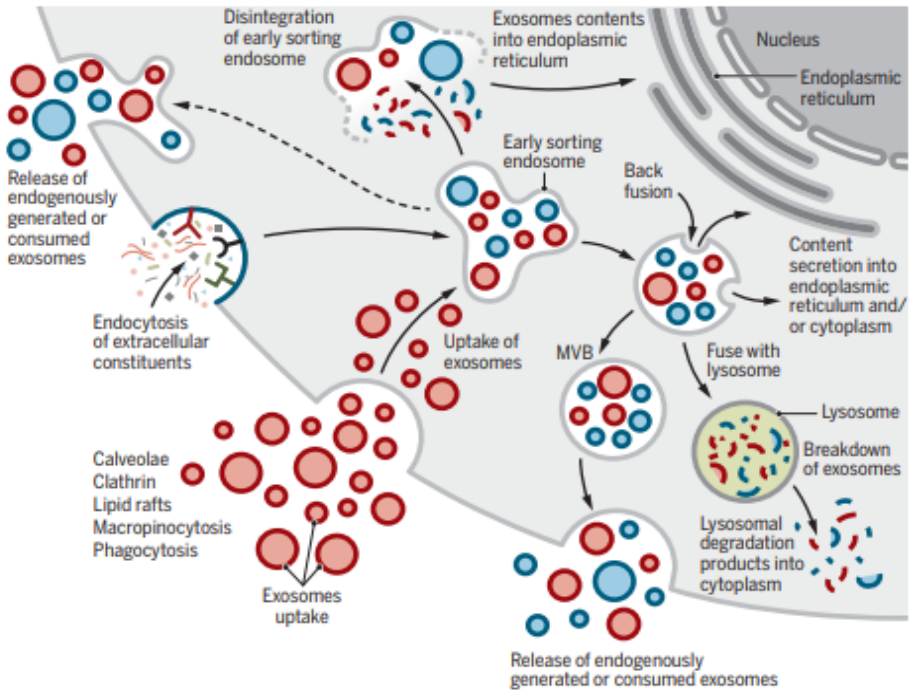

Figure 1. Intracellular process of the biogenesis of exosomes. Maturation of the early endosomes into the late endosome and the MVB, which fuses with the plasma membrane to release exosomes or fuses with lysosomes and degrades (10). *Image used with author's permission.*

1.2.1 ESCRT-dependent biogenesis of exosomes

The most commonly used pathway for the biogenesis of exosomes is the ubiquitin-dependent endosomal sorting complex required for transport (ESCRT) (15). This pathway consists of four molecular complexes (ESCRT 0-III). The ESCRT complexes are associated with MVBs (16). ESCRT-0 binds to the transmembrane protein of the endosome and loads them into the ILVs, by phosphatidylinositol 3-phosphate activation and ubiquitinated molecules present on the endosome. The ESCRT-0 contains the hepatocyte growth factor, which binds the TSG101 protein of ESCRT-I. Both complexes induce vesicle formation by invagination of the membrane. ESCRT-I is primarily responsible for sorting the cargo into the ILV. ESCRT-II also participates in cargo sorting and additionally regulates the ESCRT-III complex formation. Finally, the detachment from the membrane into the MVB is performed by ESCRT-III, which is also responsible for the sorting and concentration of the MVB cargo and further participates in the ESCRT recycling (14,16).
The biogenesis of exosomes is promoted through the cytosolic adaptive protein syntenin-1. Syntenin-1 binds syndecan and ALIX which is also involved in the ESCRT complex. These proteins support the biogenesis of IVLs, sort the cargo to those vesicles and act as a mediator between ESCRT-I and III (18).

1.2.2 ESCRT-independent biogenesis of exosomes

Even though the ESCRT pathway is described as the most important pathway of releasing cellular exosomes, there are also ubiquitin-independent pathways.
Two alternative pathways have been described. First, a ceramide-dependent mechanism, where the lipid mediator, ceramide originating from the hydrolysis of sphingomyelin, triggers the production of exosomes (19), and second the tetraspanin-dependent pathway. Research has found transmembrane proteins belonging to the tetraspanin family which have been part of cargo selection and exosome formation (20). Therefore, many different cell types follow multiple mechanisms, with diverse molecules. For example, in melanoma cells, a CD63-dependent mechanism has been detected (21). In contrast, in kidney cells the

expression of CD82 and CD9 promotes a tetraspanin-dependent and ESCRT-independent formation of exosomes (20,21).

The packing of exosomal cargo and the secretion is a well-organized mechanism based on the physiologic status of the cell. However, this mechanism is altered in response to specific stimulants like oxidative stress, infection, allergies, or inflammation which generally leads to increased exosome release. Interestingly, it has been shown that the number of released exosomes in pregnancies also increase (24). Every cell type has a unique exosomal protein and lipid composition, which could be used as a liquid biopsy reflecting the metabolic state (6).

1.2.3 Secretion of exosomes to the extracellular space and uptake into target cells

As mentioned above, there are two different outcomes for MVBs. They either fuse with lysosomes and get degraded, or fuse with the cell membrane and get released as exosomes. This transport to the membrane includes the involvement of microtubule skeleton, cortactin, actin, and RAB (ras-related in brain) proteins. Over 60 GTPases-RAB proteins are taking part in the transport and fusion of the MVB (15). It has been shown that the knockdown of various RAB proteins decreases the secretion of exosomes (25). Some of the RAB proteins are already decrypted. For example, RAB27 was found to be crucial for the transport of exosomes, including syntenin-1 and ALIX, which are assumed to be important for the docking and final fusion of the two membranes (26). Another intracellular compartment is also involved in the final fusion. After docking, the SNARE (soluble NSF-attachment protein receptor) complex with the belonging proteins SNAP(-23) and VAMP(-7,-8) supports the fusion, a mechanism regulated by Ca^{2+} (26,27).
Once released into the extracellular space, exosomes circulate in the fluid until they have reached their targeted cell. The mechanisms of exosomes interacting with the recipient cells are still not fully explored, but specific routes depending on the target cell type have been proposed. All known routes of uptake mechanisms are depicted in Figure 2 (13,28).

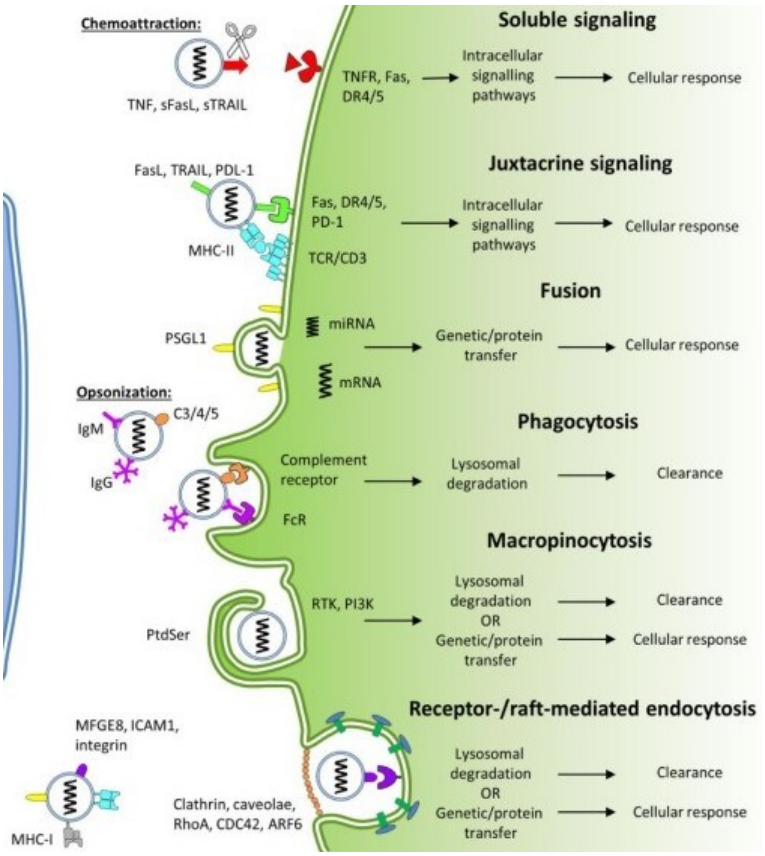

Figure 2. The different mechanisms of exosomes to enter a target cell.
The known pathways of exosomal communication are soluble and juxtacrine signaling as well as exosomal uptake through fusion, phagocytosis, macropinocytosis and receptor-/raft-mediated endocytosis. After uptake, exosomes can either induce cellular response via genetic or proteomic transfer or induce clearance by lysosomal degradation (13). *Image used with author's permission.*

Taking advantage of signaling molecules is one way of cell-to-cell communication mediated by exosomes. Further soluble and juxtacrine pathways were identified. For the soluble signaling pathway, a ligand needs to be split from the exosome by proteolysis, whereas juxtacrine signaling demands a juxtaposition of both ligand and receptor. FasL (FS7-associated cell surface antigen), TNF (tumor necrosis factor), and TRAIL (TNF related apoptosis inducing ligand) are such signal ligands (Figure 2). The process of cell fusion is not yet fully understood, it is assumed to be mediated by tetraspanins but this is still hypothesized. However, phagocytosis is an actin-mediated process that requires opsonin receptors, toll-like receptors, and scavenger receptors (13). Dendritic cells and macrophages are well-known for performing this phagocytosis but some other cells can also fulfill this task (30). Whether this process is responsible for intercellular communication or just eliminates exosomes needs further investigation. Macropinocytosis of exosomes is a PI3K (phosphatidylinositol

3-kinase) and Na^+-dependent mechanism. The plasma membrane protrudes, builds an invagination by actin filaments, and endocytoses extracellular particles and fluid (13). Like juxtacrine signaling, the receptor raft-mediated endocytosis needs associated receptors and ligands on the exosome and target cells. This process, also called clathrin-dependent endocytosis, needs the presence of clathrin and an adaptor protein complexes to start (31). All these processes induce either cellular response or clearance of the exosome and its cargo.

1.3 Markers of exosomes

To detect exosomes in biological samples, there are well-known surface proteins that can be detected by western blot measurements. All of them function through the above-described mechanisms of releasing exosomes and are expressed in the outer membrane of exosomes. These markers can be used for all types of exosomes, but there are also markers that are specific to distinct tissues. Commonly used markers are ALIX, TSG101, and syntenin-1 which are part of the ESCRT-dependent pathway of the biogenesis, and proteins of the tetraspanin family such as CD9, CD63, and CD81. The placental alkaline phosphatase (PLAP) is specific to exosomes released by the human placenta (32). For characterization of the endothelial cell released exosomes at the fetal side of the placenta, no specific marker has been described yet. To ensure specificity of exosomes, a negative control should be used. The society for extracellular vesicles recommends GRP94, also called heat shock protein 90 beta family 1 (HSP90B1), as marker protein, which is absent in exosomes but present in other extracellular vesicles (33).

1.4 The impact of exosomes

1.4.1 Immune system and metabolism

Exosomes play many different roles in intracellular communication. Their impact on the immune system is well-known, as they either stimulate or inhibit it. Further, their actions depend on their origin and cargo. The role of exosomes in immune

regulation is in the transport and presentation of antigenic peptides. They deliver cGAS STING (cyclic GMP-AMP synthase stimulator of interferon genes) which triggers the expression of inflammatory genes and a type I IFN response. Exosomes induce signaling pathways through surface ligands and manipulate gene expression through exosomal miRNA (10).

For example, antigen-presenting cells (APCs) release exosomes that contain major histocompatibility complexes (MHC) class I and II, which can induce specific immune responses by inactivating CD4+ and CD8+ T-cells. Interestingly, the direct T-cell stimulation of APCs is more efficient than the exosomally induced one (34). Nonetheless, a mice experiment had shown tumor growth reduction after a single intradermal APC-derived-exosome injection (35). Another study shows that dendritic cell-derived exosomes promote T-helper response and IFNg production (34). Antigen presentation was also detected in macrophage-derived exosomes in the context of bacterial infection wherein the exosomes may enhance the antibacterial immune response and afterward influence the adaptive immune response (36). Exosomes also influence the immune response through miRNA. These noncoding RNAs, transferred as exosomal cargo, regulate biological processes and gene expression by directly interacting with the recipient cell. On the one hand, miRNA can be exchanged between dendritic cells, repressing gene expression and mediating dendritic cell maturation (37). On the other hand, exosomes play a role in cancer development by mediating the interaction in the tumor stroma by supporting migration, metastasis, and treatment resistance (38). As shown by Ding et al. 2015, tumor-derived exosomal miR-212-3p circumvents the immune system by down-regulating the MHC-II transcription factor RFXAP (regulatory factor X associated protein) in dendritic cells (39). Additionally, bacteria and viruses themselves emit exosomes, which were detected in humans and have an impact on disease progression (40). Using the exosomal pathway supports the pathogens in entering the host by pretending to be endogenous (41). This could be a benefit in developing exosome-based drug therapy. Interestingly, no EV-mediated immune reaction in whole blood transfusion is known, even though they carry trillions of EVs (10). However, exosomes are not only interesting for immune system-related or infectious diseases. Many studies show that the onset and course of critical illnesses correlate with the level of circulating exosomes (42). Besides

nucleic acids and proteins, exosomes carry metabolites. A mouse model by Deng et al. 2009 shows how exosomes released by adipose tissue promote insulin resistance mediated through its recipient cell (macrophages) (43).

1.4.2 Exosomes in pregnancies

Pregnancy is a vulnerable and well-balanced process. It is a masterstroke to sustain and readapt the requirements of the mother and fetus. The embryo in the stadium of a blastocyst needs to be implanted into the maternal endometrium to receive nutrition. The embryonic cells themselves are a combination of maternal and paternal DNA, which means that a foreign genotype enters the maternal system. Immune tolerance towards the embryo is a condition for successful implantation. As mentioned earlier, exosomes can influence the immune system, which implies their crucial role in maintaining pregnancy. After implantation, the placenta is built of maternal and fetal cells, providing fetal nurturing, communication, and a blood barrier. The most important part of forming the placenta is the transformation of the uterine spiral arteries. Research has shown the influence of exosomal intracellular communication in these processes, which can be broke down in the following aspects.

The first interesting finding is that the concentration of exosomes in the whole blood of pregnant women is 50-fold higher than in non-pregnant women and increases with gestational age. The placenta releases 12-25% of these exosomes. Levels of exosomes are linked to the BMI of the mother. Pregnant women with a higher BMI show decreased total exosomes in maternal circulation (44). Potentially, one function of these exosomes is to adapt the maternal metabolism in response to fetal demands.
BMP (Bis(monoacylglycerol)phosphate) in exosomes could be another reason for the increased exosome release. Progesterone, which is at high levels during pregnancy, stimulates BMP which is considered to take place in the ILV formation. Interestingly, BMP is also assumed to prevent HIV transmission from mother to fetus (45).

Further, trophoblast-derived exosomes show the ability to induce monocyte migration to differentiate into tissue macrophages which secrete chemokines and cytokines, to promote the growth and survival of the trophoblast (46).
These exosomes also carry HLA-G molecules. Their immunosuppressive function contributes to the immune tolerance towards the fetus (47). Exosomes also carry bioactive lipids like prostaglandin E2 (PGE2). PGE2 is immunosuppressive and can be part of the implantation process. Another lipid (15d-PGJ2) carried by exosomes is a ligand to the transcription factor PPARɣ (peroxisome-proliferator-activated-receptor), which controls placental development and parturition (48). PPARɣ is also known to control the expression of syncytin-1 which leads to the hypothesis that it is also part of syncytiotrophoblast formation (7). Further, fusogenic exosomal molecules are phosphatidic acids and the aforementioned BMP (49). Another important role is the remodeling of the spiral vessels to generate a constant blood flow to support the fetus and the perpetuation of the pregnancy. Exosomes released by trophoblastic cells seem to depend on oxygen and glucose concentration. They increase under hypoxic conditions. These exosomes stimulate proliferation and the invasion of the extravillous trophoblast (EVT). By entering the decidua, they connect with it and adapt to the environment, by altering the spiral vessels to achieve a constant blood supply (50). The migration of vascular smooth muscle cells (VSMC) plays a key role in spiral artery remodeling. EVT cell-derived exosomes promote this migration through a novel EVT-VSMC exosomal communication pathway (51), which decreases in the third trimester (52). It is also shown that the embryo releases exosomal miRNAs and vascular endothelial growth factor A (VEGFA) to adjust blood flow (53). Depending on the oxygen level, especially in hypoxic conditions, placental exosomes showed stimulation of vasculo-angiogenesis (54). Another research on exosomal-promoted angiogenesis shows how maternal and umbilical-derived exosomes from healthy pregnancies support human umbilical vein endothelial cells (HUVEC) in proliferation and migration. 258 miRNAs were elevated in both types of exosomes (52).

It seems that these exosomes carry all the required molecules to promote cell fusion, initiate syncytiotrophoblast formation, and perform the spiral vessel remodeling. This leads to the assumption that exosomes could also play a role in

pathological pregnancies like fetal rejection, growth restriction, and preeclampsia (47).

To prevent pregnancy, placental exosomes reduce T-cell response by inhibiting CD3-ζ and Janus Kinase 3 (JAK3) expression and activating caspase-3. This causes apoptosis in target cells which may be due to their correlation with FasL and PD-L1 (programmed death-ligand 1) (44). Compared with preterm pregnancies, the T-cell suppression in term labor is significantly higher (55).

MiRNAs are assumed to communicate immune tolerance towards the fetus as well. MiR-517a-3p which belongs to the MI57 family, inhibits the NO/cGMP/PRKG1 pathway in maternal immune cells. It was shown that miR-517a-3p decreases after delivery and couldn't be found in non-pregnant women (56).

There is much more research to be done on the role of exosomes in pregnancies and it can be assumed that this will be done. It shows the influence of exosomes during the entire vulnerable process of pregnancy. It also includes their possible role in the emergence of pregnancy pathologies and their potential for treating pregnancy diseases like preeclampsia.

1.5 Preeclampsia

Preeclampsia is a common and potentially harmful gestational syndrome with a vascular defect in placental vessels. It displays in the form of hypertension and proteinuria and can lead to seizures and death in its end stage, called eclampsia. 2% of all pregnancies in Europe are diagnosed with preeclampsia. 15% of these pregnancies deliver preterm, 28% of the fetuses show intrauterine growth restriction, and 10-15% of maternal deaths are associated with this disease. Additionally, preeclampsia causes higher morbidity for mother and child.

Despite this frequent occurrence and the medical consequences, the actual causes of preeclampsia are not yet fully explored (57).

1.5.1 Risk factors, clinical signs, and symptoms

In opposite to the pathophysiology, the risk factors and the clinical appearance of PE are well known. Risk factors can be subclassified in general and pregnancy-related factors. The S2k-guideline of AWMF (Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften e.V.) names the following: autoimmune diseases, anti-phospholipid syndrome, BMI > 30 kg/m^2, age > 40 years, preexisting diabetes mellitus or nephrological diseases, primum parity, chronic hypertension, preeclampsia in a previous pregnancy, family history and Afro-American ethnicity. Pregnancy-related risk factors are gestational diabetes, multiples, invitro fertilization, and a high resistance of the arteria uterine (notching) (58).

Preeclampsia is clinically defined as a new-onset hypertension with a blood pressure > 140/90 mmHg after the 20th week of pregnancy, pathological serum markers and one further organ manifestation. In general, the kidney is damaged, which leads to proteinuria (> 300 mg/dl) or high serum creatinine. It can further present liver damage with high serum liver enzymes, or neurologic dysfunction (including headaches, seizure, and hemorrhagic stroke), as well as left ventricular hypertrophy, pulmonary edema, and hematologic disturbance (showing thrombocytopenia, and disseminated intravascular coagulopathy). On the fetal side, PE can cause intra-uterine growth restriction (59). Preeclampsia can further be classified into early-onset, in which the gestational age is lower than 34 weeks, and late-onset.

1.5.2 Pathophysiology

Since the termination of pregnancy is known as the only curing therapy for preeclampsia, it is conclusive that the origin of PE is located in the placenta itself. The condition for a successful implantation is a healthy and functional trophoblast. During placentation, trophoblast cells invade the decidua, the inner third myometrium, and the vessel wall to induce the remodeling of the spiral arteries. These junctional vessels from the uterine artery perform the decidual blood supply.

For this adaptive process, they lose smooth muscle cells and dilatate 5-10-fold at the vessel endings. Furthermore, arterio-venous shunts are built in the myometrium. These changes cause vessels without vasomotoric responsiveness and enable a steady low-pressure blood flow (60).

In preeclampsia, these spiral arteries do not dilate and cause a blood flow with a higher pressure around the villi (Figure 3). This leads to reduced contact time between the blood and the villous surface, which causes a low oxygen supply towards the fetus. These arteries also show a higher sensitivity to vasoconstrictive triggers, which cause chronic oxidative stress and placental ischemia. On the maternal side of the placenta, this oxidative stress causes the release of vasoactive substances like cytokines, oxidized lipids, free radicals, and soluble vascular endothelial growth factor 1. These factors are responsible for vascular and organ damage. There are two possible explanatory approaches for the misguided implantation (61). One is genetical determination: studies have shown several preeclampsia-linked genes (62). The other suggests a defective maternal immune system that misses the fetoplacental unit. An immune cell overshoot causes the secretion of TNFα which provokes apoptosis of the extravillous cytotrophoblast (63). Lower levels of the human leucocyte antigen HLA-E and HLA-G in the serum of women with PE emphasize this hypothesis (64), as well as the high levels of soluble FMS-like tyrosine kinase 1 (sFlt-1) - an antagonist for VEGF and placental growth factor (PlGF), and a common PE marker (65). Even though these research findings try to explain the onset of preeclampsia, the underlying pathomechanisms still remain unclear.

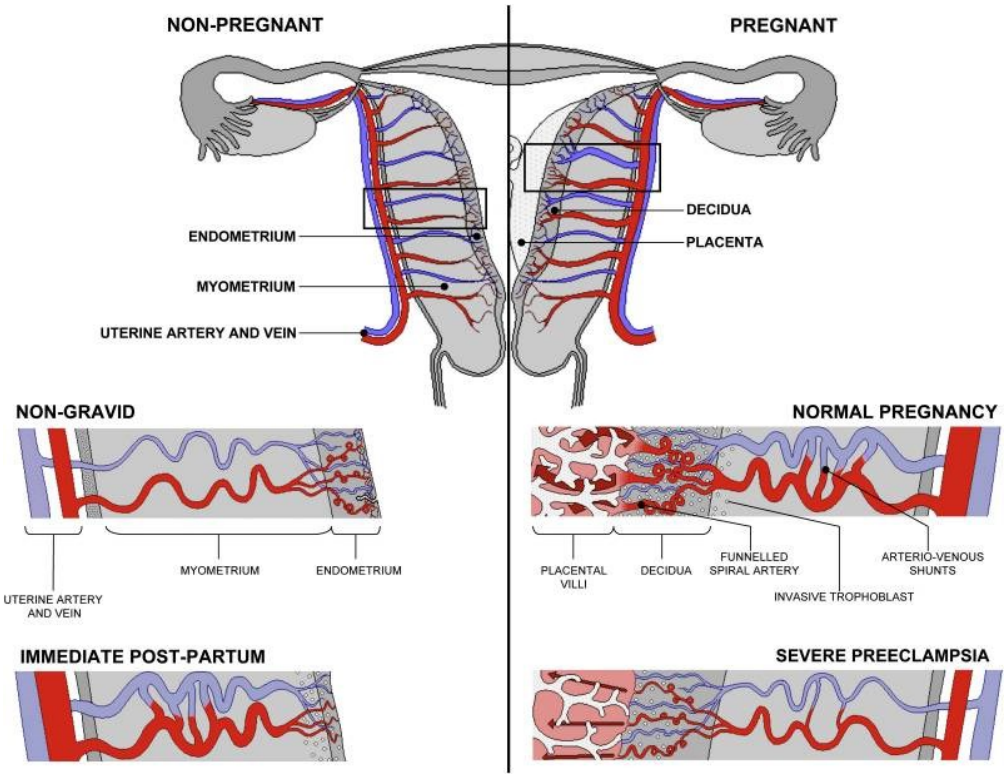

Figure 3. Development of spiral arteries in healthy and preeclamptic placentas (60)**.**
*Image used with author's permission***.**

1.5.3 The potential role of exosomes in preeclampsia

Different studies show correlations between the cell-to-cell communication of exosomes and preeclampsia. Various parts of exosomes were observed to express differently in PE than in healthy pregnancies. Most of the research has concentrated on the maternal side of the placenta, where exosomes were analyzed from serum. As mentioned earlier, exosome levels increase during pregnancy. But it was also shown that there is no significant difference between the number of exosomes in healthy versus preeclamptic pregnancies (66). The difference originates in the cargo, namely miRNAs and proteins of exosomes. Exosomes derive from trophoblastic cells to the maternal blood and transmit miRNA that potentially communicates angiogenesis, and in case of PE apoptosis, to recipient tissues. Research has shown that miR-155 is overexpressed in PE, which affects angiogenesis by inhibiting eNOS, which promotes the disease (67,68). Trophoblast inhibition and dysfunction are also induced by miR-29b and miR-136 (68). Sandrim

et al found miR-885-5 significantly increased in PE and suggest it as a possible marker for liquid biopsies (69). Further, hypoxia leads to an increase of miR-210, which suppresses the invasion of trophoblasts (70).

Another interesting finding is the difference between early and late-onset preeclampsia observed by Pillay et al. Levels of placenta-derived exosomes are increased in early-onset preeclampsia and decreased in late-onset PE. The miRNA profile is different as well: In case of late-onset preeclampsia, miR-297 and miR-375 were identified which suggests a dysregulated glucose homeostasis (71).

In addition to the microRNAs, there are exosomal proteins that are associated with PE. Exosomes from preeclamptic placentas show a high expression of the aforementioned sFlt-1 and the soluble endoglin (sEng) which is involved in vascular remodeling due to its interacting with thyroglobulin antibodies (TGAB) (72,73). Both proteins can reduce the growth of HUVECs and reduce vascular migration. SEng is a suitable preeclampsia marker since it is rarely found in healthy pregnancies (72).

The immunomodulatory impact of exosomes is also part of the pathophysiology of PE. Exosomes transmit signals to the maternal immune system and influence pro-inflammatory factors (74). Decreased placental protein 13 - transmitted by exosomes, leads to increased inflammatory response and could promote PE (75).

An exosome-independent study shows a significantly elevated level of CD81 in syncytiotrophoblasts in patients with early-onset preeclampsia. Since CD81 is a tetraspanin embedded in the exosomal surface and a typical exosome marker, this result might be nonetheless relevant (76).

Overall, little is known about the fetal side. A research group from Canada examined villous-derived exosomes and found that syncytin-2 is reduced in preeclamptic pregnancies. Syncytin-2 plays a key role in the embryonal implantation (77). Another group focused on umbilical cord plasma and found that preeclamptic exosomes inhibit the proliferation and migration of HUVECs compared to healthy pregnancy exosomes. They figured out a correlation with the significantly decreased HMGCS1 (3-hydroxy-3-methylglutaryl-CoA synthase 1) (78).

It remains unclear if exosomes can pass the placenta barrier or if communication occurs through molecule transmission. Some research argues that, as PLAP has been detected in fetal serum, exosomes must pass the placenta. But there is still no conclusive evidence to support this hypothesis.

Summing up these findings on exosomes and PE, further research is required for a better understanding of the pathophysiology and to find approaches for prevention, diagnosis, and therapy.

2 Hypothesis and aims

There is little knowledge on fetoplacental-derived exosomes, their characteristics, and whether protein cargo differs from exosomes isolated from placental vessels of preeclamptic women. This research focuses on the surface proteins of exosomes derived from fetoplacental vessels. Preeclampsia is a vascular disease that originates in the formation of placental vessels. Obviously, the vascular exosomes could show differences as well. The main differences on the maternal side were detected in the cargo, whether the microRNAs or the proteins. But as Shen et al figured, CD81 (a surface marker of exosomes) is elevated in the blood of PE women, and affects endothelial cell dysfunction, there could also be differences in the surface proteins of preeclamptic endothelial-derived exosomes.

As mentioned earlier, exosomal surface proteins are mainly involved in the formation of exosomes and the sorting of their cargo. We wanted to figure out if these surface proteins, which we use as common exosome markers, are affected in PE pregnancies as well. We hypothesize that surface proteins from preeclamptic endothelial-derived exosomes differ significantly from those of healthy pregnancies. This investigation shall serve as a first indication of whether there is an impact of exosomal surface proteins in this disease.

Due to the small sample size, we used both types of vessels (veins and arteries) for the analysis. To guarantee that any possible differences between the two vessels do not affect the significance of our results, we first compared the exosomal proteins of veins to those of arteries. Furthermore, we decided to compare the size distribution of the exosomes as well as the total amount of protein levels.

3 Material and methods

3.1 Study set-up

To address the objectives, we planned a pilot study with several samples to compare the total amount of the investigated surface proteins. Therefore, exosomes were isolated and then examined in a western blot series. The obtained results should serve as a first indication for further studies.

We took pre-cultured and pre-characterized cell isolations from our lab. In a time-lapse of six months, all utilized endothelial cells were thawed, grown, isolated, and examined. To guarantee comparability, a standard operation protocol was applied. Furthermore, all isolations were characterized by size distribution in the Nanosight instrument and later probed by immunoblotting, including negative controls.

3.2 Study group

For this study, 21 different biological samples of fetoplacental vessels (n = 10 arterial and n = 11 veins) were isolated and analyzed. Subjects of used placentas were aged between 24 and 46 years and had a preconception BMI range of 18.1 to 33.9 kg/m^2. Six of the women gave spontaneous births, 14 delivered by cesarean section, one was not recorded. The pregnancies ended between days 210 and 286. Seven pregnancies were diagnosed with PE. Six of these had early onset preeclampsia (< 33+6 pregnancy week), and one was classified as late-onset preeclampsia (> 34+0 pregnancy week). For this study, we did not differentiate between early and late-onset preeclampsia.

All included women signed informed consent and this study was approved by the local ethics committee (EK No. 29-319 ex 16/17). Anonymized clinical data was provided by the department of obstetrics at the Medical University of Graz.

For the statistical analysis, pregnancy progression and vessel type were used as independent parameters; due to the small study size we assume a non-gaussian distribution.

Table 1. Distribution of used samples in targeted groups.
P.c.: pregnancy course; PE: preeclamptic pregnancies; nonPE: healthy pregnancies.

		Vessel		
		Arterial	Venous	Total
P.c.	nonPE	7	7	14
	PE	3	4	7
Total		10	11	21

3.3 Isolation of primary human placental endothelial cells

Human placental vascular endothelial cells (HPVEC) from veins and arteries were isolated from chorionic vessels of term placentas. According to published protocols, the isolation of primary cells was performed (79,80). The placental tissues were donated by women who gave birth at the LKH Graz. Written informed consent was obtained (EK No. 29-319 ex 16/17).

Briefly, one chorionic plate-vein and -artery (each at least 3 cm long and 1 to 3 mm in diameter) were cut out of the tissue and washed in Hanks´ Balanced Salt Solution (HBSS, Gibco, #14175053). The vessels were cannulated with an infusion cannula (Vasofix Safety Pur 20G, 1.1 mm, Braun, #4269110S-01) and flushed with 5 ml HBSS. To detach the endothelial cells, a pre-warmed 37 °C collagenase/dispase solution (diluted to 0.5 mg/ml with HBSS, Roche, #11097113001) plus penicillin/streptomycin (10000 µg/ml, Gibco) was used. After disposing of the first three drops, 20 ml of enzyme solution was rinsed through the vessel for six to eight minutes (5 ml/ two minutes) into a 10 ml tube with fetal calf serum (FCS) (Hyclone, #SH30070.03) to gather the necessary material. The digested suspension was centrifuged at 900 rpm (rounds per minute) at room temperature for seven minutes. The pellet was resuspended in 1 ml of Endothelial Cell Growth Medium MV Kit (PromoCell, #C-22120) and 10% serum of pregnant woman (pooled and heat-inactivated from our hormone laboratory staff) and transferred into a well of a 12-well plate coated with porcine skin gelatine (Sigma-Aldrich). The plate was incubated at 37 °C at 12% oxygen and the media was changed on day two or three. When the cells reached up to 50% confluence, they were transferred into a 12.5 cm^2

flask and cultivated in standard cultivation media, Endothelial Cell Growth Medium MV Kit (PromoCell, #C-22120) with 0.1% gentamicin (Gibco, #15750037). Cells were split into 25 cm^2 and finally into 75 cm^2 flasks at 90% of confluence. After the first splitting of the primary cells, arterial and venous endothelial cells were separated and cultivated at different oxygen concentrations (arterial cells: 12% oxygen; venous cells: 21% oxygen). After approximately eight weeks of cell cultivation, samples were frozen in freezing media containing 20% DMSO (Dimethylsulfoxid) (Sigma- Aldrich or Serva) and stored in liquid nitrogen for further experiments (79).

3.4 Cell culture

Cells used for this study were kindly provided by W. Brandl. They were thawed in a thawing device (ThawSTAR®) and cultivated in a 75 cm^2 flask with standard cultivation media. HPVEC were grown under standard culture conditions (37 °C, 12% or 21% oxygen, 5% CO_2, humidified atmosphere). Cell culture media was changed twice a week and cells were split at 90% confluence. All used flasks and well plates were coated with 1% gelatine. Cells were expanded into three 175 cm^2 flasks per isolation to a confluence of approximately 70%.

48 hours before the isolation of the exosomes, cells were washed with HBSS and challenged with Endothelial Cell Growth Basal Medium MV containing 5% exosome-depleted FCS (Gibco, #A2720801), human epidermal growth factor (PromoCell), hydrocortisone (PromoCell), and gentamicin (Gibco, #15750037).

3.5 Isolation of exosomes

The total supernatant of all three flasks per isolation (approximately 70 ml) was collected in two 50 ml tubes. The cells were harvested for cell counting, as described below, and used for protein and RNA isolation.

To isolate the exosomes, the cell culture medium was centrifuged several times, at 4 °C. First, to discard the remaining cells and cell debris, the medium was centrifuged at 500 x g for 10 minutes in the Allegra X12R benchtop centrifuge. The

obtained supernatant was transferred into 50 ml tubes and again centrifuged at 500 x g for 20 minutes to get rid of apoptotic bodies and smaller fragments.
For the extraction of MVs, the supernatant was centrifuged in an Optima XE-90 ultracentrifuge (Rotor Type 70 Ti, Beckman Coulter) at 12 000 x g for 30 minutes. The pellet was kept for measuring the size of the MVs and stored for further experiments. To remove particles larger than 200 nm, the supernatant was filtered through a 0.2 µm filter (Millipore Steriflip with a vacuum pump). For the last isolation step, the filtrate was concentrated with a Pierce™ Protein Concentrator PES (100 000 MWCO, Thermo Fisher Scientific) to ~ 4 ml volume by centrifugation. The concentrate was split into two ultracentrifuge tubes (Beckman Coulter, #342413) and filled up with filtered PBS (0.02 µm, Medicago). The sealed tubes were centrifuged at 100 000 x g for 22 hours at 4 °C in the ultracentrifuge.
The obtained pellet contains exosomes, the supernatant was discarded. One pellet was resuspended in 200-250 µl (depending on cell counts) filtered PBS for the Nanosight (NS300 instrument, Malvern Panalytikal GmbH) measurements (further called ‘sample’) and the other one in 700 µl QUIazol (Qiagen) to isolate mRNA for further experiments.
Five samples, vesicles from venous (n = 3) and arterial (n = 2) cells from non-preeclamptic pregnancies, were isolated and then measured in the Nanosight instrument, by another lab-employee earlier. These samples have been thawed for western blot analysis.

3.6 Cell counting

One of the three 175 cm^2 flasks was used for counting the cells. After removing the supernatants for the isolation of exosomes, the cell monolayer was washed with HBSS and incubated with 4 ml TrypLE Select (Gibco, # 12563011) for three minutes under standard conditions. After the cells detached from the flask, the fluid was re-suspended in an additional 16 ml standard medium and collected in a 50 ml tube. To count the cells, Casy TT® System (OLS, Omni Life Science) was used. Cells were suspended in a defined buffer solution (CASYton). 100 µl of the cell suspension was added to 10 ml CASYton. The cells generated an electrical impulse

while passing a probe (81). Data was presented in 'cell counts per ml', where dilution was already considered.

3.7 Size distribution of vesicles

To prove whether the isolation of different vesicles corresponds to literature data, the size of vesicles was determined by the Nanosight NS300 and analyzed by NTA3.2 Software. The Nanosight is a light scattering system, based on a laser, to analyze the presence, concentration, and size distribution of all types of nanoparticles with a diameter between 10 nm to 1000 nm (82). The samples were diluted 1:50 in 0.02 µm filtered PBS, in total 1 ml. For the measurements, the blue laser module with 488 nm was used. For the performance the following standard settings were used. ‘SOP Standard Measurement’: syringe pump flow: 50; camera level: 14; five measurements of 60 seconds each (number of frames: 1498); temperature: 23-24 °C; viscosity: water.

The produced results were a concentration per ml, the mean and mode of size distribution as well as the associated graphs. As negative control in every process, a sample of the used 0.02 µm filtered PBS was measured, like the matching microvesicle sample to proof size range. In between every measurement, the pump system was cleaned with sterile water and ethanol.

Afterward, the undiluted samples were frozen at -70 °C until they were required for the immunoblotting.

3.8 Protein concentration measured by BCA Protein Assay

To spread an equal amount of protein per sample on the gel, the total protein concentration per sample was determined with a Pierce BCA™ Protein Assay Kit (Thermo Fisher) and with the help of an absorbance microplate reader. A standard curve was determined by bovine serum albumin (BSA) standard samples. Technical triplicates were utilized. As reference, seven standard samples, with given protein concentrations, were added to every measurement (125, 250, 500, 750, 1000, 1500, and 2000 µg/ml). The experiment was performed according to the manufacturer’s

protocol. 5 µl sample and 200 µl prepared working reagent were used per well. Solutions were mixed on a plate shaker for 30 sec and then incubated at 37 °C for 30 minutes. Before measurement, the plate was cooled down to room temperature. The plate reader measured the absorbance at 562 nm. The average result was calculated as µg/ml. The accuracy has been proved by using the standard curve.

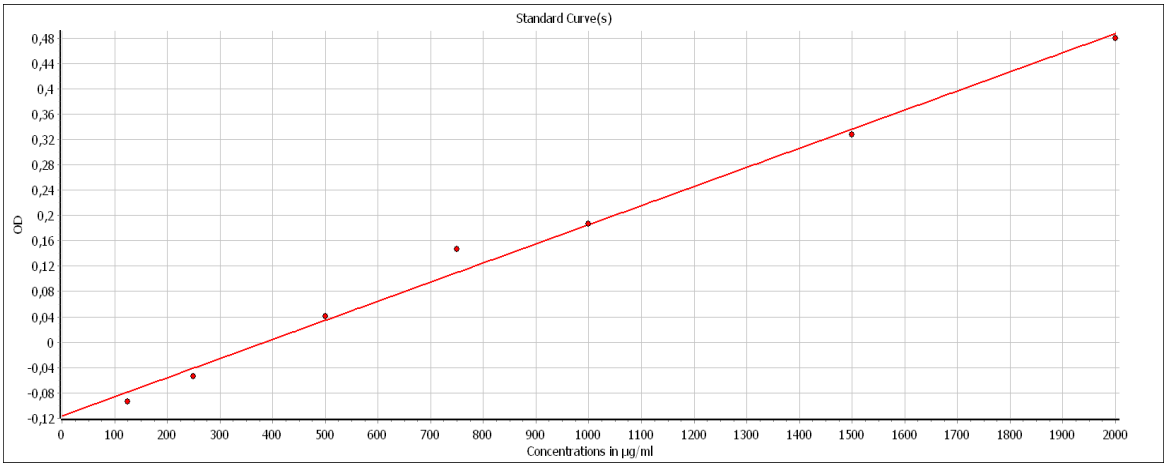

Figure 4. BCA protein assay standard curve (example).

3.9 Western Blot

3.9.1 Preparation

The samples for examination were diluted with PBS to equilibrate the protein concentration for each sample (approximately 6 µg/ml). For western blot analyses, the samples were further diluted with 4 x Laemmli buffer (Biorad). The samples' proteins were denatured by boiling at 95 °C for five minutes. Afterward, they were vortexed and stored on ice for future use. For gel electrophoresis, a Bio-Rad Mini Protean Tetra Cell System and 10-well 4-20% Mini-PROTEAN® TGX™ Precast Protein Gels with 50 µl slots (#4561094), were used. Each gel was loaded with two PageRulers, a placental tissue protein standard, and seven samples of 36 µl each. The electrophoresis was performed with 110 V and 400 mA for around 70 minutes. To transfer the result to the Trans-Blot® Turbo™ Mini Nitrocellulose membrane, the Bio-Rad Trans Blot Turbo Transfer System was used.

3.9.2 Labeling

No-Stain™ protein labeling was performed, to refer the targeted proteins to the total protein concentration afterward. Therefore, the membrane was pre-washed twice, each with 20 ml ultrapure water for two minutes. Then, the membrane was incubated with the No-Stain™ Membrane Labeling Solution (20 µl of No-Stain™ Activator, 20 µl No-Stain™ Derivatizer, and 10 µl (1 x) No-Stain™ Labeling Buffer) for 10 minutes on a rotating platform with 60 rpm. To discard the labeling solution, the membrane was washed with 20 ml ultrapure water for another 10 minutes. The washing water was replaced every two minutes. For imaging, the Fusion FX by VILBER and the Fusion FX Software (EvolutionCapt FX6 17.03), with its Fluorescence mode (AlexaFluor 488™) at high sensitivity, were used.
Ponceau staining of the gels was performed to control the loading and transfer efficiency of the proteins.
After imaging the marker proteins (NoStain™), the membrane was stained again for five minutes in Ponceau solution on the shaker. For de-staining and imaging, the membrane was washed with distilled water until just the protein bands were visible. Imaging was performed with the Blot Marker mode of the Fusion FX. Finally, the membrane was cleaned with TBE-T (Tris-Borate-EDTA Buffer + 0.1%Tween®).
Both described protocols were performed for each western blot. Due to comparability reasons of the images, all results were referred to Ponceau staining.

3.9.3 Blocking and antibody incubating

Membranes were blocked for one hour with 10 ml of 5% milk on the shaker before being incubated with the first antibodies. To save resources, membranes were cut as shown in Figure 5 within the expected weight areas.
After blocking, the cut membranes were incubated in 2-4 ml of the primary antibody solution (Table 2) in a plastic bag, which was sealed without any air bubbles, and incubated overnight on the shaker at 4 °C.

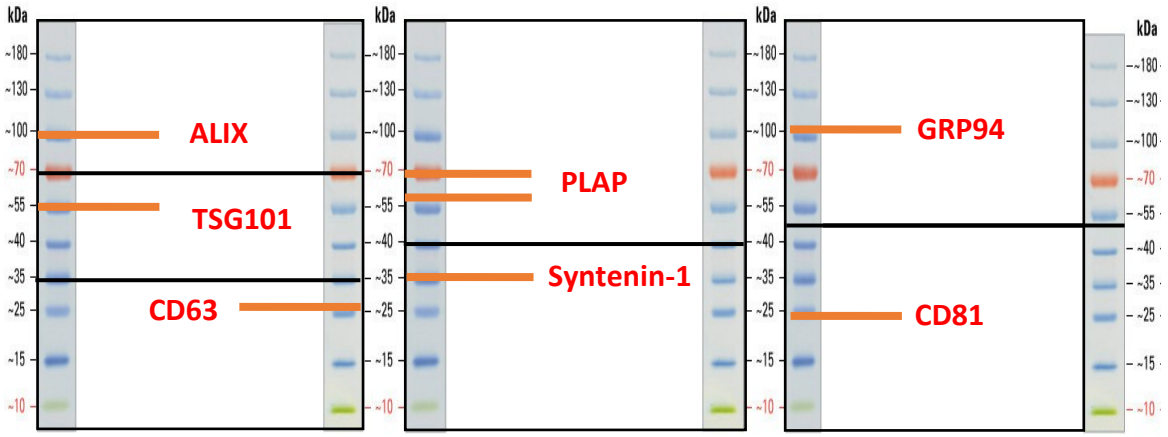

Figure 5. Splitting of the membranes (black lines) in the specific protein kDa-range.

Table 2. Applied primary antibodies.

name	company/cat	Host	dilution	size
CD63	*Abcam/ ab8219*	*mouse*	*1:500*	*30 kDa*
CD63	Proteintech/ 25682-1-AP	rabbit	1:300	26 kDa
CD81	GeneTex/ GTX101766	rabbit	1:500	20/26 kDa
Syntenin	Abcam/ ab 19903	rabbit	1:750	32 kDa
TSG101	Abcam/ ab125011	rabbit	1:1000	46 kDa + 31 kDa
ALIX	Covalab/ pab0204	rabbit	1:500	91-93 kDa
GRP94 (HSP90B)	Santa Cruz/sc-393402	mouse	1:500	58-70 kDa
PLAP	Abcam/ ab133602	rabbit	1:500	55/ 70 kDA

To remove the excessive antibodies, membranes were washed with TBE-T on the shaker for one hour, with a buffer change every 10 minutes. Afterward, membranes were incubated with the secondary antibody (Table 3) on the shaker for another hour. Then, the washing process was repeated.

Table 3. Applied secondary antibodies.

Name	Host	company	Cat
mouse IgG	goat	BIORAD	170-6516
Rabbit IgG H&L	goat	BIORAD	170-6515

3.10 Imaging and quantification

Imaging was done with the Fusion FX by VILBER and the Fusion FX Software (EvolutionCapt FX6 17.03) with the Chemiluminescence Mode and Add Marker. The membranes were prepared with the Thermo Scientific™ SuperSignal™ West Pico solution (1:1 of Stable Peroxide Solution and Luminol/Enhancer solution) at room temperature for five minutes. Afterward, membranes were placed between two clear sheets that all air bubbles had been removed from carefully before imaging. The system automatically calculates the exposure time and takes a picture of the blot marker. For some membranes, the exposure time had to be adapted.
To relate the protein concentration of the blots to the Ponceau stained membranes, the volume of the bands was measured by using the Fusion FX Western Blot Quantification.

3.11 Statistics

Statistics were performed with SPSS and graphs were created with GraphPad Prism (Version 9). With a total sample number of 21 and only seven in the smallest (PE) group, which represents a non-Gaussian distribution, groups were compared with the Man Whitney U Test, with a CI of 95%, and significance was considered $p < 0.05$.

4 Results

4.1 Cell culture

Cells were grown under standard conditions and expanded into three 175 cm^2 flasks per isolation to a confluence of approximately 70% before their isolation and analysis. Arterial endothelial cells showed the typical mature phenotype and cobblestone pattern. Venous endothelial cells showed a characteristic spindle-shaped growth with a juvenile phenotype and fibroblastoid swirling patterns as described by I. Lang et al 2008 (80). Endothelial cells isolated from a PE placenta presented similar characteristics as control cells but were characterized by overall slower growth. Some thawed preeclamptic cells couldn't be used due to growth restriction, thereby not achieving confluence, or losing shape. This already is the first indicator of a disrupted angiogenesis in preeclamptic cells.

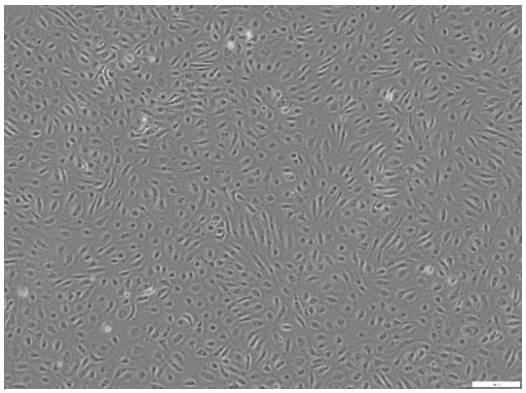

Figure 6. Arterial endothelial cells (ECA 159).

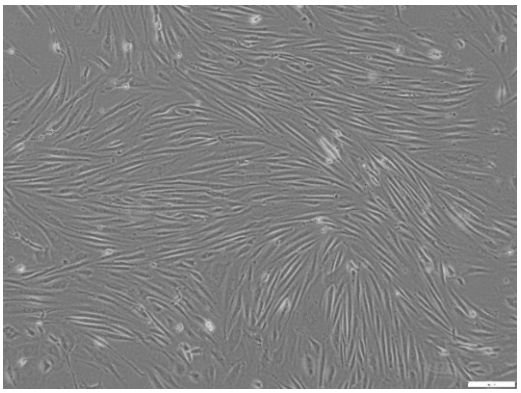

Figure 7. Venous endothelial cells (ECV 150).

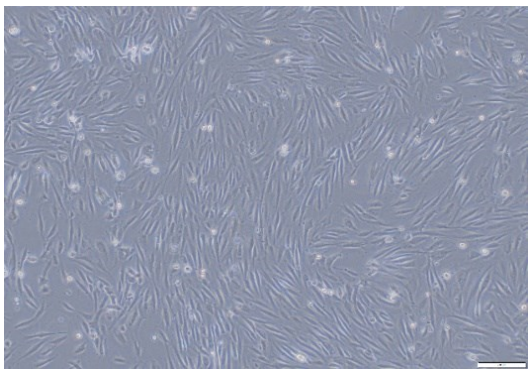

Figure 8. Preeclamptic venous endothelial cells (ECV 17PE).

4.2 Western blot

Exosomes of HPVEC from preeclamptic and healthy pregnancies were isolated by multiple centrifugation steps. Isolated exosomes were further characterized by immunoblotting and probed by typical surface proteins (ALIX, TSG101, syntenin-1, CD81), the trophoblast-specific-protein PLAP and the glucose-related protein GRP94 as a negative control, to ensure that the tested exosomes originate from the cultured fetoplacental endothelial cells. All selected proteins were detected in the expected size range (Figure 11). To prove purity, a western blot of the used medium and a microvesicle sample were performed for all proteins.

In addition, the exosomal surface protein CD63 was probed but both used antibodies (Abcam, ab8219; Proteintech 25682-1-AP) did not exhibit specific bands, which is why this marker was skipped for further analysis. These results were compared between cell types (arterial, venous) and pregnancy course (preeclampsia, healthy). Two different types of staining were used (No-Stain™ Protein labeling; Ponceau staining. Figures 9, 10) to check the protein load of respective gels. Due to a lack of consistency in the No-Stain™ labeling, the Ponceau staining was used as the reference staining for this analysis.

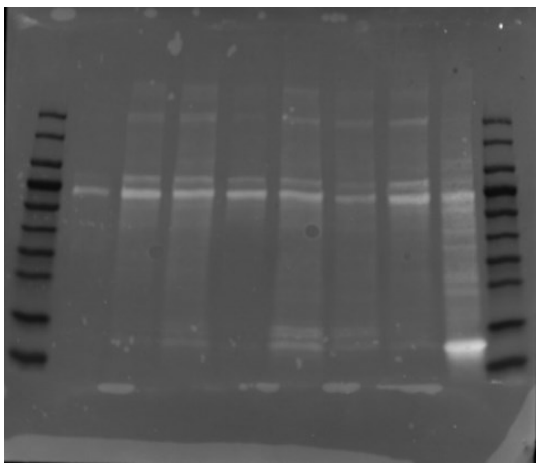

Figure 9. No-Stain labeling.

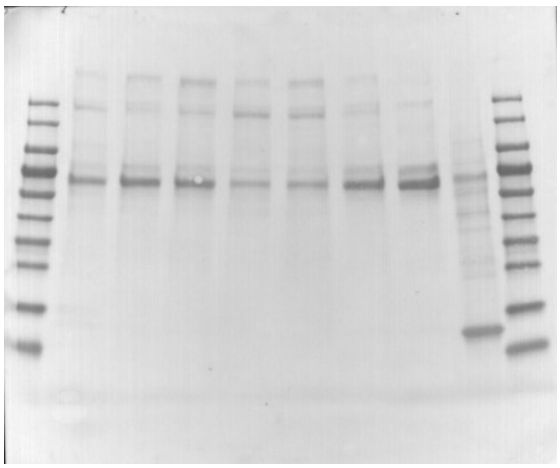

Figure 10. Ponceau staining.

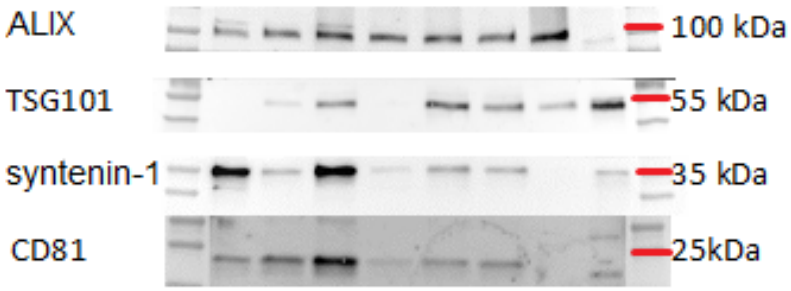

Figure 11. Western blot for selected ESCRT proteins. (A: artery; V: vein; (V): vein not used for statistical analysis; APE: preeclamptic artery; VPE: preeclamptic vein; M: medium; MV: microvesicles; PT: placental tissue; PR: page ruler)

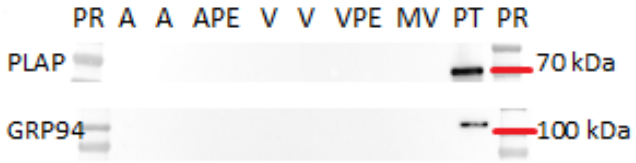

Figure 12. Western blot negative control – only show up in placental tissue (PT). (A: artery; V: vein; APE: preeclamptic artery; VPE: preeclamptic vein; M: medium; MV: microvesicles; PT: placental tissue; PR: page ruler)

4.2.1 Differences of exosomes from arterial and venous endothelial cells

To compare the exosomal surface proteins of fetoplacental veins and arteries, a total of 21 samples were analyzed (arterial: n = 10, venous: n = 11).

4.2.1.1 ALIX

The ESCRT-associated protein ALIX was detected in all samples used for this study. The differences in the expression of ALIX between placental arterial cell-derived exosomes and venous cell-derived exosomes is not statistically significant ($p = 0.132$). The western blot shows a wide variety of ALIX densities in both arterial (A) and venous (V) derived exosomes (Figure 14). As shown in Figure 13 there is a wider range in the amount of ALIX in the venous samples compared to the arterial.

ALIX was also detected in the medium, which wasn't expected. In a second western blot, the medium was analyzed again, and ALIX was still detected. A reason for this could not be found in the literature. As shown in chapter 4.3 the medium presented particles of exosomal size in the Nanosight experiment. This could an explanatory approach for the occurrence of ALIX. Since the same medium was used for all samples, it does not have any impact on the results. There is no ALIX in the microvesicle sample. The slight bar in the placental tissue is not at the right kDa area so it can be considered an artifact.

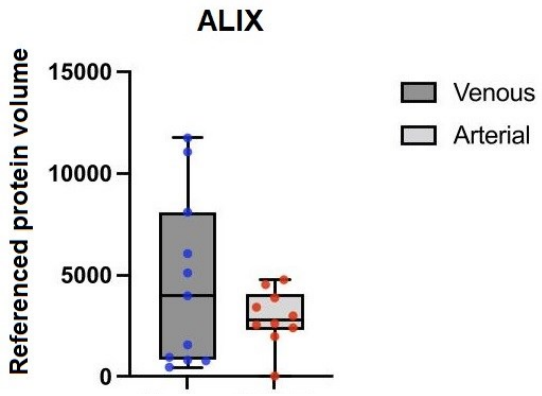

Figure 13. Volume distribution of ALIX in vessel group analysis.

Table 4. Statistical analysis of ALIX in vessel group.

	Alix
Mann-Whitney U	33,000
Wilcoxon W	88,000
Z	-1,549
Asymp. Sig. (2-tailed)	,121
Exact Sig. [2*(1-tailed Sig.)]	,132[b]

a. Grouping Variable: Vessel

b. Not corrected for ties.

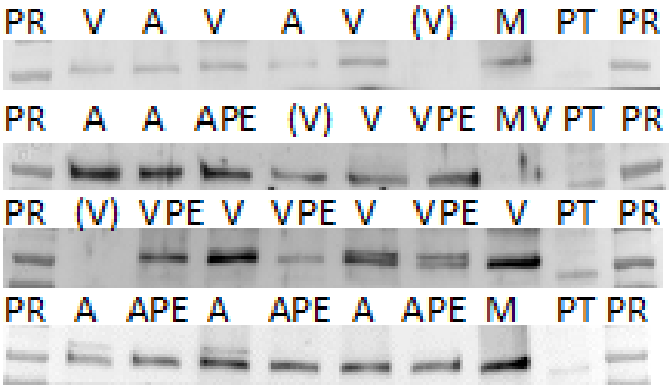

Figure 14. Western blot – ALIX. (A: artery; V: vein; (V): vein not used for statistical analysis; APE: preeclamptic artery; VPE: preeclamptic vein; M: medium; MV: microvesicles; PT: placental tissue; PR: page ruler)

4.2.1.2 TSG101

The exosome secretion-associated marker TSG 101 shows less intense densities in the western blot (Figure 16). Especially in the second western blot, it seems like there is almost none. The volume quantification and reference to the total protein confirmed its presence in all used samples. With p = 0.314, there is no statistical significance between the expression of TSG101 in arterial and venous-derived exosomes. The figure shows that the venous exosomes show a substantially wider range of TSG101 amount (Figure 15) with an overall more even distribution. No TSG101 was detected in the medium or in the microvesicles, but there are intense bands in the placental tissue (Figure 16).

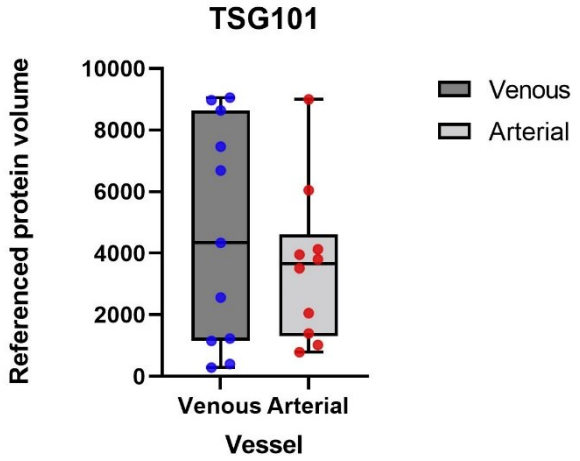

Figure 15. Volume distribution of TSG101 in vessel group analysis.

Table 5. Statistical analysis of TSG101 in vessel group.

	TSG
Mann-Whitney U	40,000
Wilcoxon W	95,000
Z	-1,056
Asymp. Sig. (2-tailed)	,291
Exact Sig. [2*(1-tailed Sig.)]	,314[b]

a. Grouping Variable: Vessel

b. Not corrected for ties.

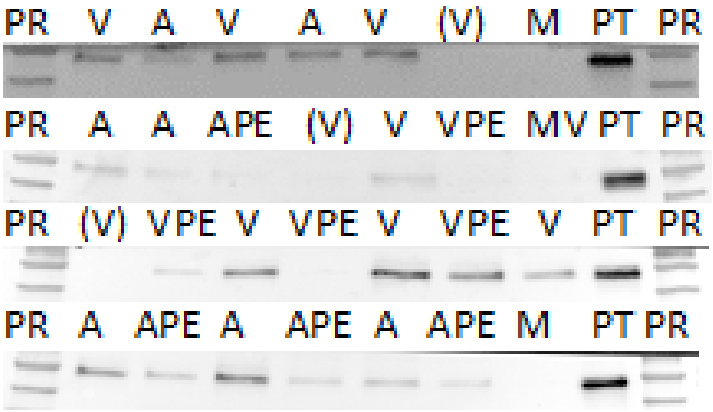

Figure 16. Western blot - TSG101. (A: artery; V: vein; (V): vein not used for statistical analysis; APE: preeclamptic artery; VPE: preeclamptic vein; M: medium; MV: microvesicles; PT: placental tissue; PR: page ruler)

4.2.1.3 Syntenin-1

The universal exosome marker syntenin-1, which is part of the exosomal biogenesis, was detected in all analyzed samples. No statistically significant difference in the compared arterial and venous exosomes could be determined (p = 0.468), although the arterial exosomes show a wider range in the protein amount as shown in Figure 17. There is a slight band at the microvesicle sample and no detection in the medium. Syntenin-1 is also found in the placental tissue (Figure 18).

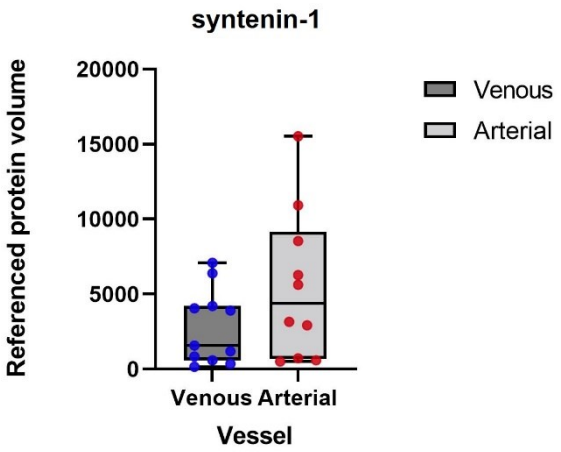

Figure 17. Volume distribution of syntenin-1 in vessel group analysis.

Table 6. Statistical analysis of syntenin-1 in vessel group.

	Synt
Mann-Whitney U	44,000
Wilcoxon W	110,000
Z	-,775
Asymp. Sig. (2-tailed)	,439
Exact Sig. [2*(1-tailed Sig.)]	,468[b]

a. Grouping Variable: Vessel

b. Not corrected for ties.

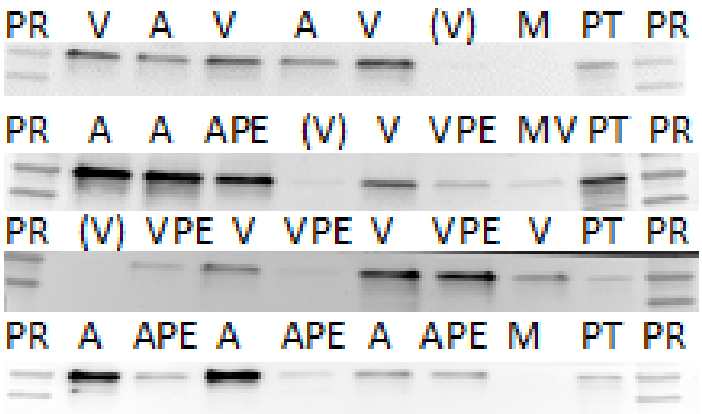

Figure 18. Western blot – syntenin-1. (A: artery; V: vein; (V): vein not used for statistical analysis; APE: preeclamptic artery; VPE: preeclamptic vein; M: medium; MV: microvesicles; PT: placental tissue; PR: page ruler)

4.2.1.4 CD81

The tetraspanin CD81 was present in all analyzed samples. It is also slightly positive in the microvesicle sample. In the placental tissue, there is a light bar, but it is not in the right kDa area, so it could also be an artifact (Figure 20). With a p = 0.282 (CI 95%), the statistical analysis shows no significant difference in the expression of CD81 between venous and arterial endothelial-derived exosomes.

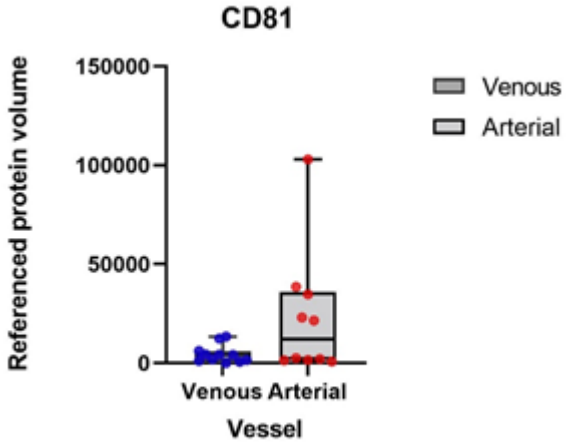

Figure 19. Volume distribution of CD81 in vessel group analysis.

Table 7. Statistical analysis of CD81 in vessel group.

	CD81
Mann-Whitney U	39,000
Wilcoxon W	105,000
Z	-1,127
Asymp. Sig. (2-tailed)	,260
Exact Sig. [2*(1-tailed Sig.)]	,282[b]

a. Grouping Variable: Vessel

b. Not corrected for ties.

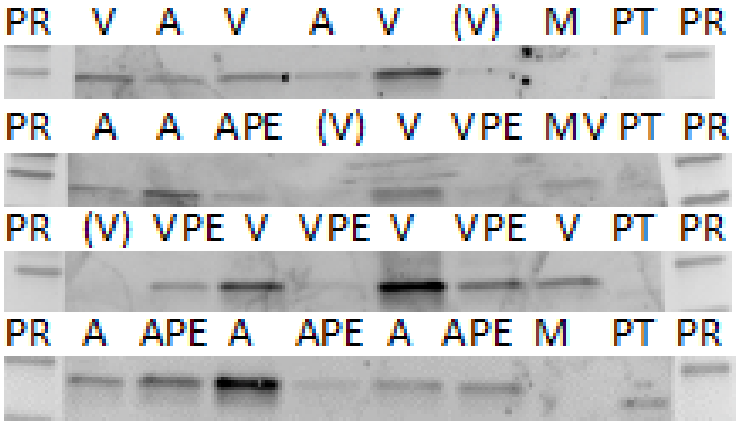

Figure 20. Western blot - CD81. (A: artery; V: vein; (V): vein not used for statistical analysis; APE: preeclamptic artery; VPE: preeclamptic vein; M: medium; MV: microvesicles; PT: placental tissue; PR: page ruler)

All in all, there is no statistical evidence for a difference in the expression of the proteins ALIX, TSG101, syntenin-1, and CD81 in arterial and venous fetoplacental endothelial-derived exosomes. So, the following comparison on surface proteins is not limited in its validity by the fact that arteries and veins were not analyzed separately.

4.2.1.5 Preeclamptic and non-preeclamptic exosomes

To compare the exosomal surface proteins of fetoplacental vessels depending on the pregnancy course, a total of 21 samples were analyzed (preeclamptic: n = 7; non-preeclamptic: n = 14). For this comparison, statistics were performed for each protein (ALIX, TSG101, syntenin-1, CD81).

4.2.1.6 ALIX

ALIX was detected in all analyzed samples (Figure 14(see above.)). The western blot results of the ALIX quantity in exosomes from preeclamptic (PE) placentas and non-preeclamptic (nonPE) show no statistical significance with p = 0.172 (CI 95%). As shown in Figure 21 there is a wider range in the non-preeclamptic group.

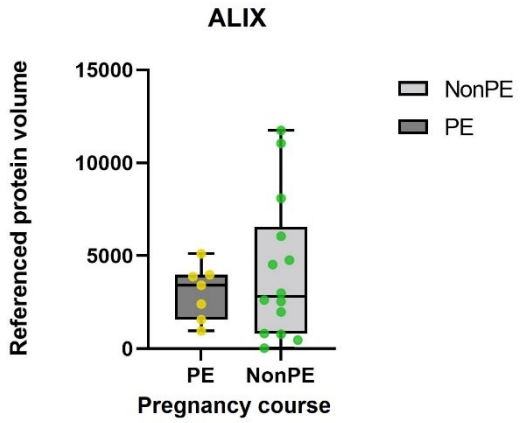

Figure 21. Volume distribution of ALIX in pregnancy course group analysis.

Table 8. Statistical analysis of ALIX in pregnancy course group.

	Alix
Mann-Whitney U	30,000
Wilcoxon W	58,000
Z	-1,417
Asymp. Sig. (2-tailed)	,156
Exact Sig. [2*(1-tailed Sig.)]	,172[b]

a. Grouping Variable: PE

b. Not corrected for ties.

4.2.1.7 TSG101

The exosomal protein TSG101 was found to be expressed in all analyzed samples. As shown in Figure 22 the distribution of the protein amount over the two groups (PE and nonPE) seems very similar. The test statistics confirm with p = 0.128 (CI 95%) that there is no significant difference between those groups.

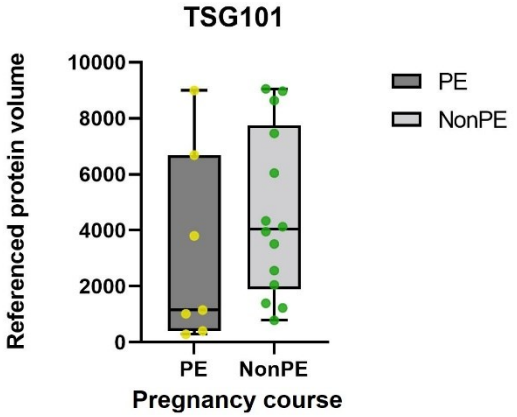

Figure 22. Volume distribution of TSG101 in pregnancy course group analysis.

Table 9. Statistical analysis of TSG101 in pregnancy course group.

	TSG
Mann-Whitney U	28,000
Wilcoxon W	56,000
Z	-1,567
Asymp. Sig. (2-tailed)	,117
Exact Sig. [2*(1-tailed Sig.)]	,128[b]

a. Grouping Variable: PE

b. Not corrected for ties.

4.2.1.8 Syntenin-1

The only protein that shows statistical significance, with a p-value of 0.031 (CI 95%), in the comparison of the preeclamptic and the non-preeclamptic group is the biogenesis-associated protein syntenin-1. As Figure 23 shows, most of the preeclamptic placental-derived exosomes have a smaller amount of syntenin-1 than the non-preeclamptic. It is also visible on the western blot in Figure 18 that the concerned bands in the preeclamptic sample are less distinct.

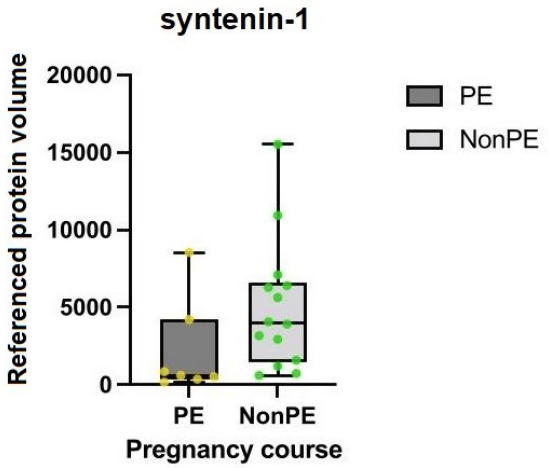

Figure 23. Volume distribution of syntenin-1 in pregnancy course group analysis.

Table 10. Statistical analysis of syntenin-1 in pregnancy course group.

	Synt
Mann-Whitney U	20,000
Wilcoxon W	48,000
Z	-2,164
Asymp. Sig. (2-tailed)	,030
Exact Sig. [2*(1-tailed Sig.)]	,031[b]

a. Grouping Variable: PE

b. Not corrected for ties.

4.2.1.9 CD81

The membrane protein CD81 was found in alle analyzed samples. As already shown in the vessel comparison, it is far less distinct in most of the samples and shows no significant difference between the preeclamptic and the non-preeclamptic group, with a p-value of p = 0.400 (CI 95%). Yet in the preeclamptic group there are two samples with a noticeably higher expression of CD81 (Figure 24).

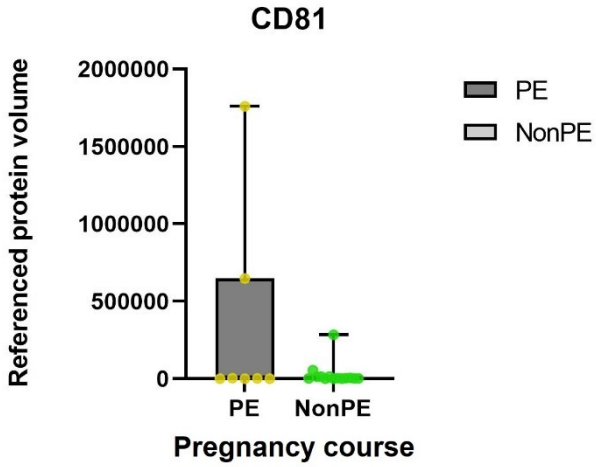

Figure 24. Volume distribution of CD81 in pregnancy course group analysis.

Table 11. Statistical analysis of CD81 in pregnancy course group.

	CD81
Mann-Whitney U	37,000
Wilcoxon W	65,000
Z	-,895
Asymp. Sig. (2-tailed)	,371
Exact Sig. [2*(1-tailed Sig.)]	,400[b]

a. Grouping Variable: PE

b. Not corrected for ties.

Summing up the results, there is no statistical difference in the expression of ALIX, TSG101, and CD81 in endothelial-derived exosomes from the given samples of preeclamptic and non-preeclamptic fetoplacental vessels. However, the expression of syntenin-1 – with a p = 0.031 – shows a statistical significance.

4.3 Size distribution of exosomes

The Nanosight instrument measured the isolated exosomes' size distribution and concentration. This procedure was used for purity proof on the one hand and to display the difference in the size distribution of both groups (vessel type and pregnancy course) on the other hand. Since it could be assumed that only nanoparticles in an exosomal size range (60 - 100 nm) were contained in the samples due to the prior filtration, statistically significant deviations were not expected. For statistical analysis, the mode of all detected vesicle sizes in one probe was used. The samples showed a mode range between 71 nm and 90.9 nm.

The matching MV samples were measured to prove that the size range could function as a negative control in all processes. As shown in Figure 25 the average particle size in the MV sample is larger than 100 nm. Still, a few vesicles in the size range of exosomes were detected as well. The degree of impurity is low, but it can explain the light protein bands in the MV samples, which showed up in the western blot experiment.

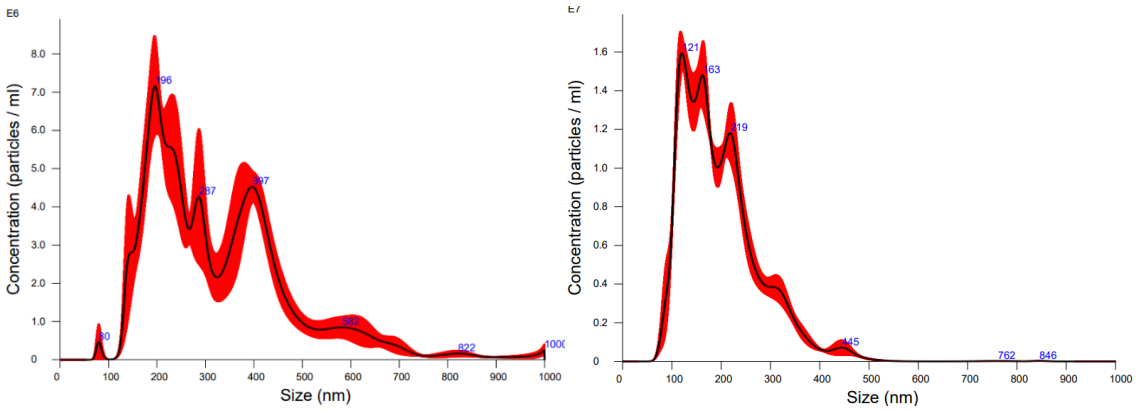

Figure 25. Graphs portraying the size distribution of arterial (left) and venous (right) derived microvesicles from the same placenta.

Furthermore, a sample of the utilized 0.02 µm filtered PBS was measured, and a probe of the medium that was used for all cell lines. As shown in Figure 26, both negative controls contain particles in an exosomal size range. Particularly the impurity of the medium is considerable. Even though this did not affect the results, since the same medium was used for all samples, these measurements still revealed a lack of purity. As shown in the western blot experiments, ALIX is the only exosomal surface protein detected in the medium. Therefore, the measured nanoparticles could also be non-exosomal.

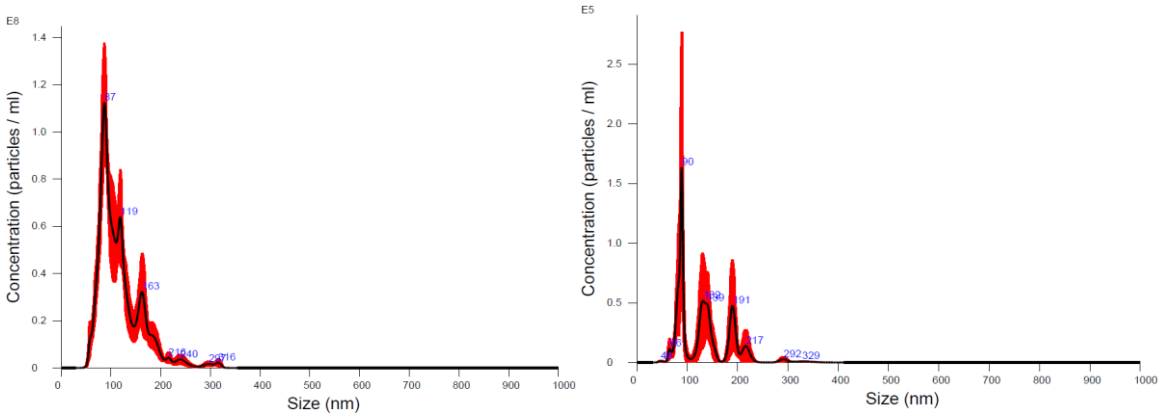

Figure 26. Graphs portraying the measured size distribution of particles in the utilized medium (left) and in an exemplary PBS probe (right).

4.3.1 Arterial and venous exosomes

As expected, the statistical analysis did not reveal a significant difference between the mode of exosome size in venous and arterial endothelial-derived exosomes. The p-value is 0.085 (CI 95%). Yet, the venous samples show a wider range of the mode. For the visual comparison of the size distribution, the curve generated by the Nanosight software was used. The given examples are from arterial and venous samples isolated from the same placentas (Figure 28, 29) The distribution appears as expected: There is one high peak of exosomes in the expected size range with a sharp decline in higher sizes. It seems like the arterial exosomes not only show one peak but also a small ‘shoulder’ peak between 100 and 200 nm which cannot be found in the venous exosomes. In other unpaired samples, this shoulder peak does not appear consistently and also appears in venous exosomes, which leads to

the conclusion that there is no difference in the size distribution based on the visual evaluation.

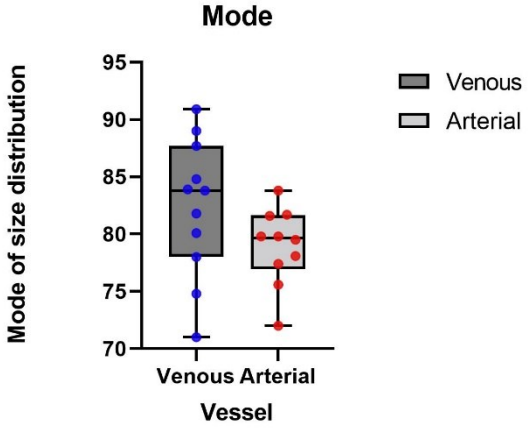

Figure 27. Mode distribution in vessel group analysis.

Table 12. Statistical analysis of mode in vessel group.

	Mode
Mann-Whitney U	30,500
Wilcoxon W	85,500
Z	-1,726
Asymp. Sig. (2-tailed)	,084
Exact Sig. [2*(1-tailed Sig.)]	,085[b]

a. Grouping Variable: Vessel

b. Not corrected for ties.

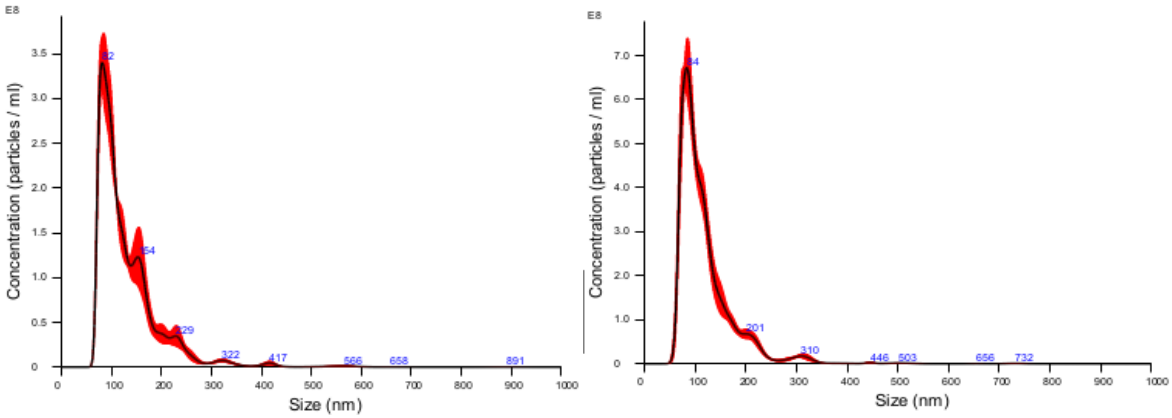

Figure 28. Graphs portraying the size distribution of arterial (left) and venous (right) derived exosomes from the same placenta.

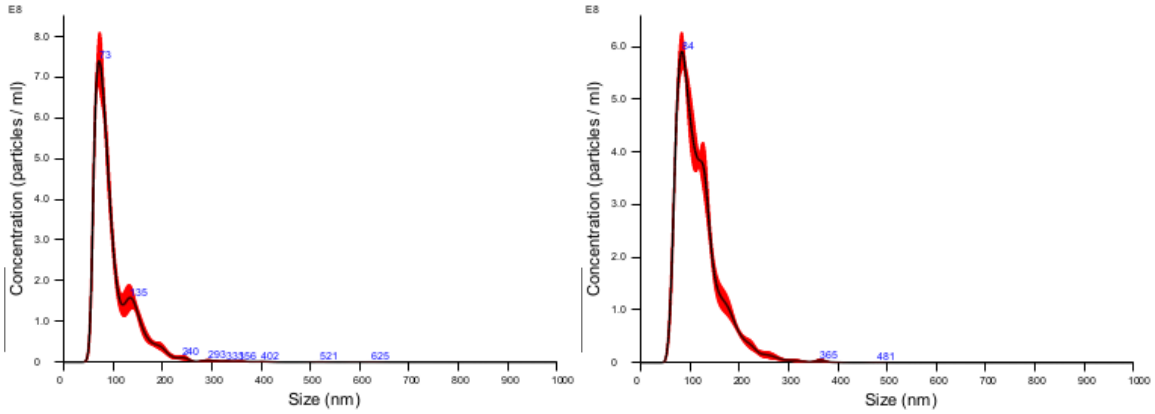

Figure 29. Graphs portraying the size distribution of arterial (left) and venous (right) derived exosomes from the same placenta.

4.3.2 Preeclamptic and non-preeclamptic exosomes

A comparison between the preeclamptic and non-preeclamptic size mode was also performed. As expected, the p-value (p = 0.224) shows no statistical significance. As shown in Figure 30, there is an even distribution, yet the size span is a little wider in the non-preeclamptic group. Comparing the curves, there is almost no difference in the size distribution (Figure 31, 32). All in all, there is no difference in between preeclamptic and non-preeclamptic exosomes as well as in the vessel variable regarding their size.

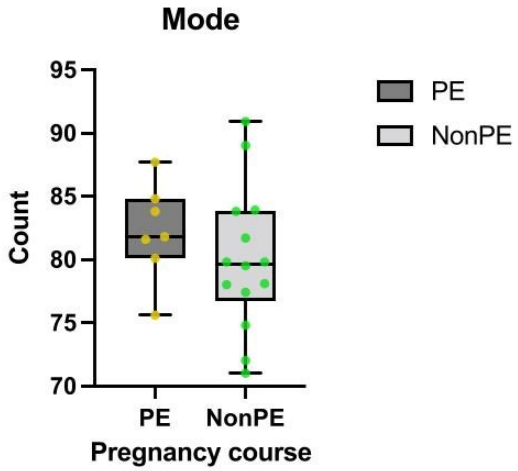

Figure 30. Mode distribution in pregnancy course group analysis.

Table 13. Statistical analysis of mode in pregnancy course group.

	Mode
Mann-Whitney U	32,500
Wilcoxon W	137,500
Z	-1,232
Asymp. Sig. (2-tailed)	,218
Exact Sig. [2*(1-tailed Sig.)]	,224[b]

a. Grouping Variable: PE

b. Not corrected for ties.

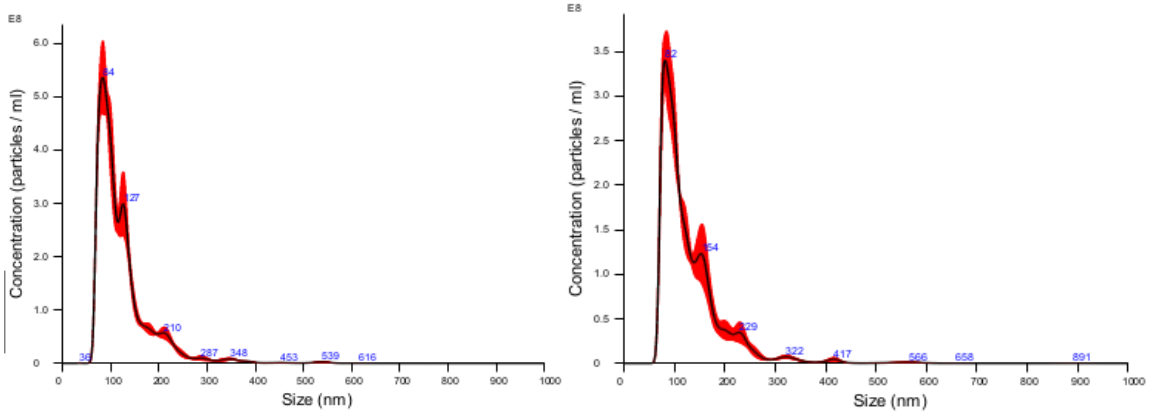

Figure 31. Graphs portraying the size distribution of arterial preeclamptic (left) and non-preeclamptic (right) derived exosomes.

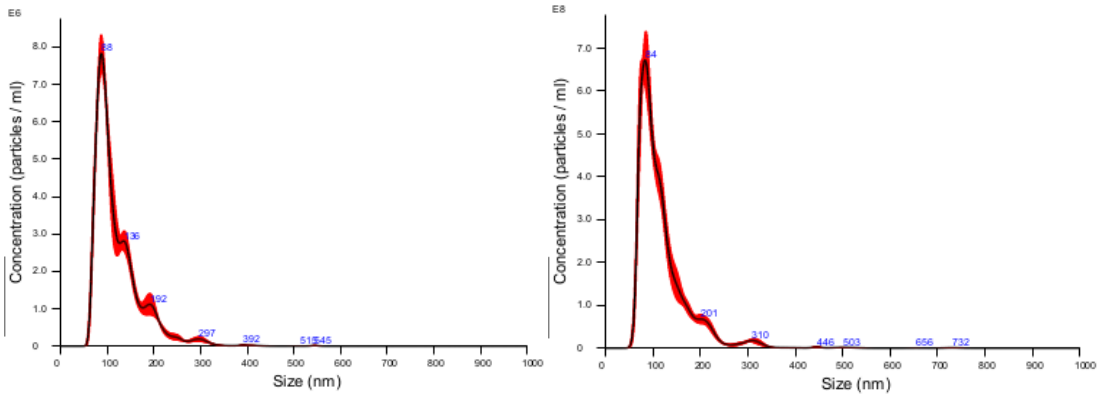

Figure 32. Graphs portraying the size distribution of venous preeclamptic (left) and non-preeclamptic (right) derived exosomes.

4.4 Protein per exosome

The last analysis should figure out if there is any difference in the protein amount per exosome (documented and analyzed in femtogram (fg)). To calculate the protein concentration per exosome, the total protein amount in µg/µl (from BCA protein assay) was divided by the exosome concentration (measured by the Nanosight software) per µl. Results were further used in fg. Statistical analysis was performed for both characteristics – vessel type and pregnancy course.

4.4.1 Arterial and venous exosomes

Regarding the protein concentration of venous and arterial-derived exosomes, there is no statistical difference in these groups ($p = 0.349$; CI 95%). As shown in Figure 33, except for one venous sample, the arterial and venous distribution and concentration ranges are almost the same. The conspicuous finding in this venous sample is caused by a low exosome concentration, with potentiation of E+05 compared to the other samples with an E+07/E+08; and a measured total protein which is similar to the other samples.

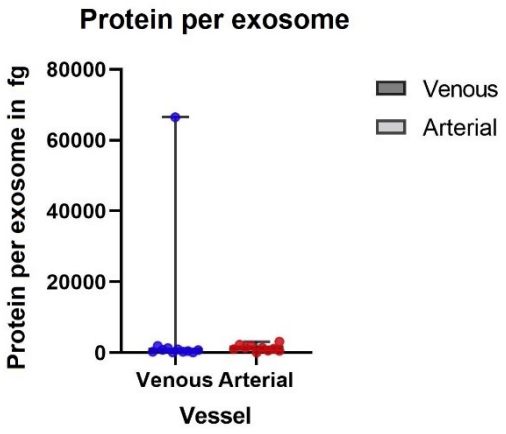

Figure 33. Protein concentration per exosome in fg compared in vessel group analysis.

Table 14. Statistical analysis of protein concentration in vessel group.

	ProtPerExfg
Mann-Whitney U	41,000
Wilcoxon W	107,000
Z	-,986
Asymp. Sig. (2-tailed)	,324
Exact Sig. [2*(1-tailed Sig.)]	,349[b]

a. Grouping Variable: Vessel

b. Not corrected for ties.

4.4.2 Preeclamptic and non-preeclamptic exosomes

The difference in protein concentrations per exosome in preeclamptic and non-preeclamptic endothelial-derived exosomes is not statistically significant. With a p-value of p = 0.128 (CI 95%), it can be assumed that there is no difference in the total measured protein depending on the pregnancy course. The above-mentioned outlier is in the preeclamptic group.

All in all, this analysis shows no evidence of a significant difference in the total protein per exosome in the compared variables.

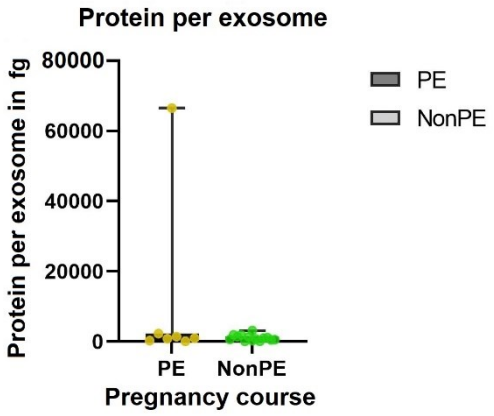

Figure 34 Protein concentration per exosome in fg compared in pregnancy course group analysis.

Table 15. Statistical analysis of protein concentration in pregnancy course group.

	ProtPerExfg
Mann-Whitney U	28,000
Wilcoxon W	133,000
Z	-1,567
Asymp. Sig. (2-tailed)	,117
Exact Sig. [2*(1-tailed Sig.)]	,128[b]

a. Grouping Variable: PE

b. Not corrected for ties.

5 Discussion

The aim of this study was to investigate core proteins of the exosomal surface in extracellular vesicles released by normal and PE primary fetoplacental endothelial cells. The hypothesis is based on the fact that the cargo of placental exosomes found in the maternal serum differ in normal and PE pregnancies. We assumed that in addition to differences in the cargo, an altered expression of endosomal sorting proteins of fetoplacental vesicles in PE may affect these vesicles within the maternal circulation to communicate the fetal needs. If so, the metabolism of the mother in late pregnancy could be triggered.

The given results do not show significant differences in the investigated surface proteins comparing venous and arterial exosomes, which confirmed our hypothesis. Generally, we found that the known ESCRT related proteins and tetraspanin CD81, which are mainly responsible for endosomal trafficking and releasing of vesicles are similarly expressed in released vesicles of arterial and venous fetoplacental endothelial cells at term.

It is of note that PE is not linked with changes of examined ESCRT protein levels, namely TSG101, ALIX, as well as the tetraspanin CD81 compared to the control samples. In contrast, syntenin-1 is less expressed in exosomes released from preeclamptic fetoplacental vessels. Syntenin-1 is also part of an ESCRT-independent pathway of biogenesis which on the other hand interacts with the ESCRT-system under some conditions. Syntenin-1 is involved in the membrane formation of exosomes, but also regulates endosomal-related sorting of the cargo inside the exosome (18). It is worth discussing why in PE, the expression of syntenin-1 is repressed in vesicles of fetoplacental endothelial cells. One may speculate that the ESCRT protein complex sorted by syntenin-1 is less required in PE, or that syntenin-1 is generally suppressed in fetoplacental preeclamptic endothelial cells by a so far unknown mechanism. Kim et al found that syntenin-1 mediates exosomes which promote cell growth, migration, and angiogenesis in lung cancer (83). If syntenin-1 has the same function in endothelial cells, it is obviously less expressed in PE, which may be connected to the disrupted migration and

angiogenesis, thereby contributing to a dysfunctional vasculature in the placenta as seen in PE. To understand the detailed impact of syntenin-1 in primary endothelial cells of the placenta at term and in PE, more detailed in vitro studies are required.

To find out whether the size and/or distribution of placental exosomes is different in PE and non-PE pregnancies, we compared obtained extracellular vesicle fractions within the groups. Mode sizes of exosomes differed neither within the vessel group nor between PE and controls. Moreover, the distribution curves as a proxy of the size of vesicles within one isolated fraction showed no abnormalities. All negative controls revealed a minor lack of purity, which could be an explanatory approach for unexpected bands in the western blot experiment. To prevent impure results, the medium could be tested in the Nanosight instrument in advance and be swapped out if it shows several nanoparticles.

To determine if there is a difference in the total amount of protein per exosome, we divided the total protein amount in µg/µl, which was measured by the BCA protein assay, by the exosome concentration per µl, measured with the Nanosight instrument. Our hypothesis that there is no significant difference, was proven. We conclude that the disease does not influence the general constitution of exosomes. This conclusion is interesting for further research on exosomes used as therapeutics.

5.1 Limitations

This study was performed with a limited number of samples, which is the biggest limitation of this pilot study. The used placentas were donated for scientific purposes by mothers who gave birth at the department of obstetrics and gynecology of the Medical University Graz and sampled and stored with a limited number of PE samples. Furthermore, some primary fetoplacental cell isolations did not grow appropriately and therefore couldn’t be used for this study. At the end, only a very low number of PE samples (n = 7) were analyzed. Given the low number of biological replicates, obtained results were not applicable for statistical normal distribution tests. Therefore, this study should be considered as a pilot.

The small sample size also limited the investigations. On the one hand, it didn't allow for a statistically reliable vessel-specific examination; on the other hand, the investigation of possible differences between veins and arteries did not allow for a differentiation between healthy and PE pregnancies. In future research, these limitations could be avoided and results could be confirmed by raising the sample size and, consequently, performing independent investigations on the different vessel types and pregnancy courses.

5.2 Prospect

The results show that syntenin-1, a protein responsible for exosomal biogenesis and cargo packing, is less expressed in fetoplacental vessel-derived exosomes in PE. This pilot study suggests conducting experiments with a higher sample number to confirm this result. Further research should focus on the function of syntenin-1 in placental endothelial cells and its impact on angiogenesis. The decreased expression of this protein in endothelial-derived exosomes could be another key to a better understanding of the pathophysiology of preeclampsia.

Most of the findings on placental extracellular vesicles in the maternal compartment focus on expression and function of miRNAs. It is well accepted that miRNAs and their capability to trigger cell functions are one of the main targets for cell-to-cell communication of exosomes. Studies on fetoplacental vesicles should also be carried out on the role of miRNA to get deeper insights.
Overall, this study clearly identified that placental vesicles released to the fetal compartment should receive more attention, since distinct molecules of these vesicles may contribute to the pathophysiological development of the fetus.

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Appendix

1. Dataset

Protein data was received from the Fusion FX Western Blot Quantification. The given number is the measured volume of the western blot bands. The table shows raw data of ALIX, TSG101, syntenin-1, and CD81 (columns 2-5), raw data referred to the total measured protein of Ponceau staining (columns 6-9), the mode results from Nanosight tracking analysis (column 12) as well as the exosome concentration per µl (column 14). Further, the total protein measured by the BCA protein assay in µg/ml (row 13) and the total protein amount per exosome in fg (row 15).

Table 1: Abbreviations: EAC: arterial endothelial cells, ECV: venous endothelial cells; The added numbers were given from our lab in order of isolation of the primary endothelial cells; Pon: Ponceau; Ves: vessel type; V: vein; A: artery; P.c.: pregnancy course; PE: pregnancy diagnosed with preeclampsia, nPE: healthy pregnancy; TotProt: total protein; cExos/µl: exosome concentration per µl; Prot/Ex: protein (fg) per exosome

	ALIX	TSG101	syntenin-1	CD81	PonALIX	PonTSG101	Ponsyntenin-1	PonCD81	P.c.	Ves.	Mode	TotProt µg/ml	cExos/µl	Prot/ex fg
ECA 42	16877754	8976155	76996509	10670298	0,02617	0,01392	0,10928	0,02026	nPE	A	81,7	0,27	23500000	0,01149
ECV 42	7231004	22666559	25621894	9213956	0,00819	0,02566	0,03906	0,01533	nPE	V	83,8	0,59	44000000	0,01341
ECA 212	9631323	3830261	18392953	13851021	0,01985	0,00789	0,05621	0,14043	nPE	A	79,8	0,37	58100000	0,00637
ECA 150	22243251	16412788	24219717	58040964	0,04764	0,03515	0,06272	0,56049	nPE	A	79,8	0,18	31700000	0,00568
ECV 150	57477628	36516700	6780260	27980037	0,11743	0,07461	0,01578	0,06232	nPE	V	90,9	0,35	43800000	0,00799
ECA158	18805555	10452154	2976212	9972720	0,037	0,02056	0,00724	2,84019	nPE	A	72	0,38	37300000	0,01019
ECV 158	42509796	64709180	18511735	58042014	0,08093	0,1232	0,04051	0,13476	nPE	V	83,9	0,21	43700000	0,00481
ECA 159	21900889	30151709	109737413	8367946	0,02998	0,04128	0,15527	0,01612	nPE	A	79,5	0,44	31900000	0,01379
ECV 159	57571704	22575904	5196762	19823793	0,11053	0,04334	0,01191	0,04232	nPE	V	89	0,49	58300000	0,0084
ECA 1PE	15890025	41839952	2119158	23501976	0,03416	0,08995	0,00511	17,59874	PE	A	83,8	0,5	35400000	0,01412
ECA 10PE	18147157	7719391	60724391	4332646	0,02409	0,01025	0,08536	0,00835	PE	A	75,6	1,18	50900000	0,02318
ECA 18PE	17390850	17074165	2327483	10903801	0,03878	0,03807	0,00589	6,46767	PE	A	81,6	0,39	38600000	0,0101
ECV 6 PE	26355831	5953283	1706212	8865022	0,05106	0,01153	0,00353	0,01974	PE	V	87,7	0,32	481000	0,66528
ECV 10-2 PE	7414290	3106822	6155253	1732804	0,00959	0,00402	0,00849	0,0027	PE	V	81,8	0,57	20500000	0,0278
ECV 14PE	8133866	1471520	759952	5181233	0,01572	0,00284	0,00163	0,01157	PE	V	80,1	0,16	16800000	0,00952
ECV 17PE	23278205	39130838	20974649	21602155	0,03978	0,06687	0,04214	0,04409	PE	V	84,8	0,6	72100000	0,00832
ECV 20	13518801	26424576	46839943	19224638	0,0463	0,09051	0,07088	0,0661	nPE	V	74,8	0,55	27900000	0,01971
ECA 217	16129990	14070325	22321947	10891123	0,04531	0,03952	0,03157	0,03122	nPE	A	78,1	0,66	33100000	0,01994
ECV 56	20869254	30932076	39935009	15161599	0,06055	0,08974	0,0589	0,04361	nPE	V	71	0,38	42700000	0,0089
ECA 208	8765987	20798351	22603240	8017712	0,02548	0,06045	0,02928	0,02444	nPE	A	77,4	1,61	51100000	0,03151
ECV 84	26656694	29677864	45623234	43883376	0,0776	0,08639	0,06396	0,12416	nPE	V	78	0,56	202000000	0,00277

2. Clinical data

Anonymized clinical data was provided by the department of obstetrics at the Medical University of Graz. Birth procedure (column 2). Gestational age in days (column 3). Maternal age in years (column 4). BMI in kg/m^2 (column 5).

Table 2: Abbreviations: EAC: arterial endothelial cells, ECV: venous endothelial cells; The added numbers were given from our lab in order of isolation of the primary endothelial cells; PE: pregnancy diagnosed with preeclampsia; Birth Proc.: birth procedure; S: spontaneous birth; PS: birt per cesarean section; Gest.Age: gestational age; BMI bef. Preg: maternal body mass index before pregnancy. Blanc: data not available.

	Birth Proc.	Gest.Age	Age Mother	BMI bef. Preg
ECA 42	S	280	30	21,8
ECV 42	S	280	30	21,8
ECA 212	PS	286	37	21,8
ECA 150	S	280	32	19,7
ECV 150	S	280	32	19,7
ECA158	PS	269	34	21,1
ECV 158	PS	269	34	21,1
ECA 159	PS	274	24	24,4
ECV 159	PS	274	24	24,4
ECA 1PE	PS	224	38	18,4
ECA 10PE	PS	227	42	23,6
ECA 18PE	PS	243	46	19,7
ECV 6 PE	PS	244	31	20,2
ECV 10-2 PE	PS	227	42	23,6
ECV 14PE	PS	238	28	18,1
ECV 17PE	PS	249	35	18,3
ECV 20				
ECA 217	PS	274	34	18,8
ECV 56	S	210		
ECA 208	S	256	30	25,2
ECV 84	S	277	29	33,9

3. Cell count with CASY

At the end of exosome isolation one pellet was resuspended in 200-250 µl (depending on cell counts) filtered PBS for the Nanosight measurements. To count the cells, Casy TT® System (OLS, Omni Life Science) was used. Cells were suspended in a defined buffer solution (CASYton). 100 µl of the cell suspension was added to 10 ml CASYton. The cells generated an electrical impulse while passing a probe. Data was presented in 'cell counts per ml', where dilution was already considered.

Table 3: Abbreviations: EAC: arterial endothelial cells, ECV: venous endothelial cells; The added numbers were given from our lab in order of isolation of the primary endothelial cells; PE: pregnancy diagnosed with preeclampsia;

Blanc: data not available. For these samples, cell counting and Nanosight measurements were done by another lab employee earlier. Cell counts and PBS amount were not documented.

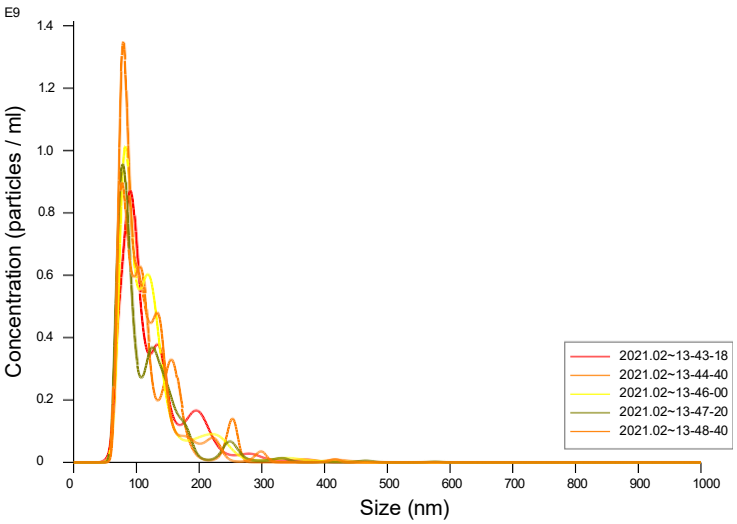
	Casy (Cells/ml)	PBS in µl
ECA 42	7,39E+04	250
ECV 42	1,22E+04	250
ECA 212	1,43E+05	250
ECA 150	1,09E+05	250
ECV 150	7,59E+04	250
ECA158	8,31E+04	250
ECV 158	1,16E+05	250
ECA 159	1,33E+04	250
ECV 159	4,39E+04	250
ECA 1PE	8,99E+04	250
ECA 10 PE	3,22E+05	250
ECA 18PE	6,37E+04	250
ECV 6 PE	8,54E+04	250
ECV 10-2 PE	9,52E+03	200
ECV 14 PE	4,72E+03	200
ECV 17 PE	1,76E+05	250
ECV 20		
ECA217		
ECV56		
ECA208		
ECV84		

4. Nanosight

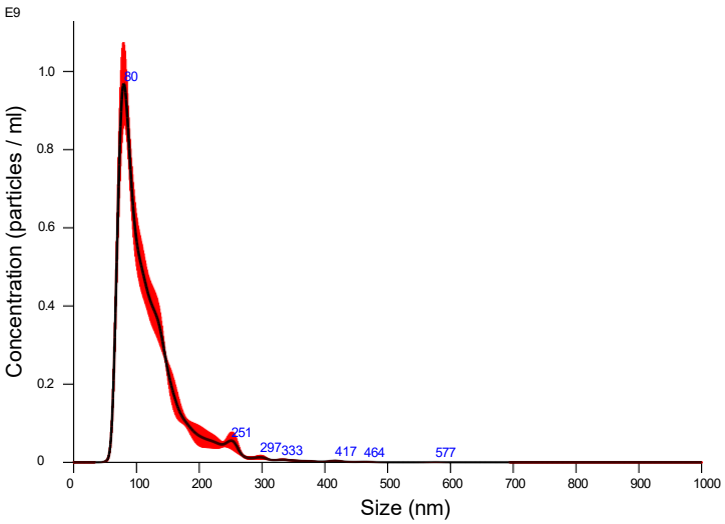
The Nanosight tracking analyses of all samples are shown on the following pages. The samples were diluted 1:50 in 0.02 µm filtered PBS, in total 1 ml. For the measurements, the blue laser module with 488 nm was used. For the performance, the following standard settings were used. ‘SOP Standard Measurement’: syringe pump flow: 50; camera level: 14; five measurements of 60 seconds each (number of frames: 1498); temperature: 23-24 °C; viscosity: water.

NANOSIGHT

2021.02.10 ECA212 2021-02-10 13-40-19



FTLA Concentration / Size graph for Experiment:
2021.02.10 ECA212 2021-02-10 13-40-19



Averaged FTLA Concentration / Size for Experiment:
2021.02.10 ECA212 2021-02-10 13-40-19
Error bars indicate + / -1 standard error of the mean

Included Files

2021.02.10 ECA212 2021-02-10 13-43-18
2021.02.10 ECA212 2021-02-10 13-44-40
2021.02.10 ECA212 2021-02-10 13-46-00
2021.02.10 ECA212 2021-02-10 13-47-20
2021.02.10 ECA212 2021-02-10 13-48-40

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 01-37-04PM 10~
Time Captured:	13:40:19 10/02/2021
Operator:	Hanna
Pre-treatment:	1:50
Sample Name:	ECA 212
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	23.7 - 23.9 ºC
Viscosity:	(Water) 0.911 - 0.916 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 13.3 - 14.6 pix

Results

Stats: Merged Data

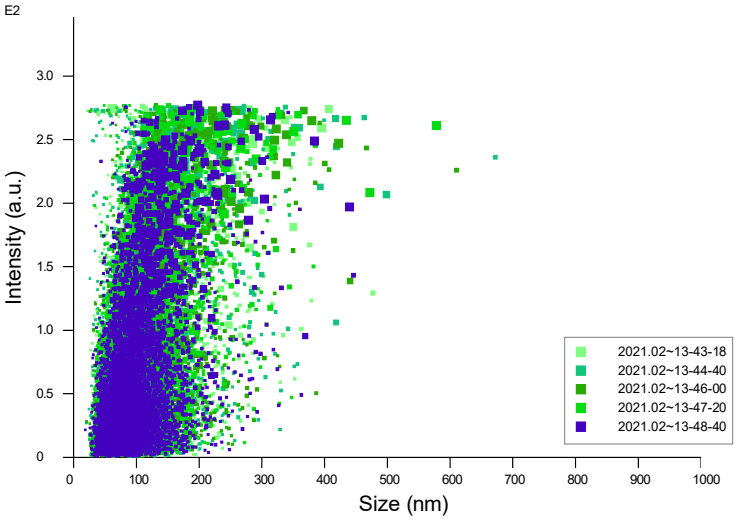
Mean:	118.8 nm
Mode:	79.8 nm
SD:	52.6 nm
D10:	73.5 nm
D50:	103.4 nm
D90:	183.7 nm

Stats: Mean +/- Standard Error

Mean:	118.9 +/- 2.0 nm
Mode:	81.3 +/- 2.4 nm
SD:	52.5 +/- 1.6 nm
D10:	73.6 +/- 0.9 nm
D50:	103.1 +/- 1.7 nm
D90:	182.3 +/- 7.0 nm
Concentration (Upgrade):	5.81e+10 +/- 2.22e+09 particles/ml
	90.5 +/- 3.3 particles/frame
	88.9 +/- 2.5 centres/frame

NANOSIGHT

2021.02.10 ECA212 2021-02-10 13-40-19



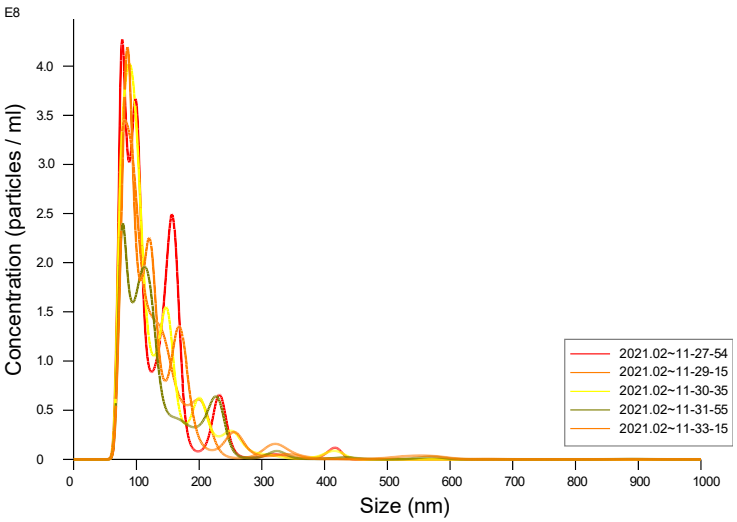
Intensity / Size graph for Experiment:
2021.02.10 ECA212 2021-02-10 13-40-19

Script Used: (Full Text):

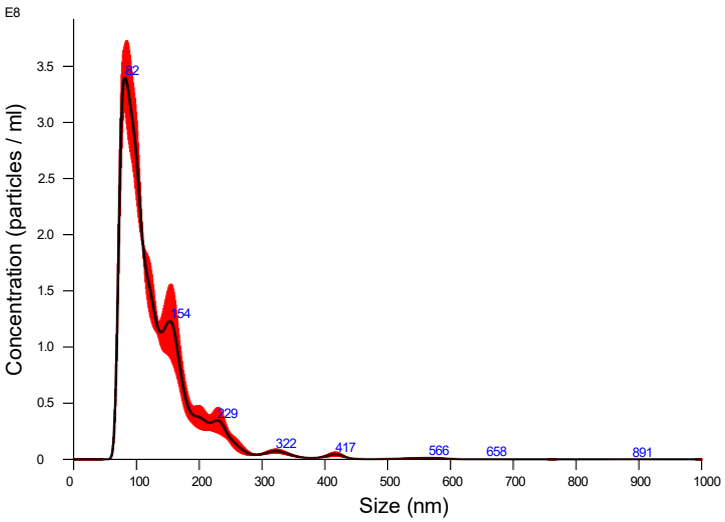
SOP Standard Measurement 01-37-04PM 10Feb2021.txt

NANOSIGHT

2021.02.25 ECA42 2021-02-25 11-25-02



FTLA Concentration / Size graph for Experiment:
2021.02.25 ECA42 2021-02-25 11-25-02



Averaged FTLA Concentration / Size for Experiment:
2021.02.25 ECA42 2021-02-25 11-25-02
Error bars indicate + / -1 standard error of the mean

Included Files

2021.02.25 ECA42 2021-02-25 11-27-54
2021.02.25 ECA42 2021-02-25 11-29-15
2021.02.25 ECA42 2021-02-25 11-30-35
2021.02.25 ECA42 2021-02-25 11-31-55
2021.02.25 ECA42 2021-02-25 11-33-15

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 11-24-12AM 25~
Time Captured:	11:25:02 25/02/2021
Operator:	Hanna
Pre-treatment:	1:50
Sample Name:	ECA42
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14 - 15
Slider Shutter:	1206 - 1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	23.4 - 23.6 ºC
Viscosity:	(Water) 0.918 - 0.922 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	100

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 12.4 - 14.1 pix

Results

Stats: Merged Data

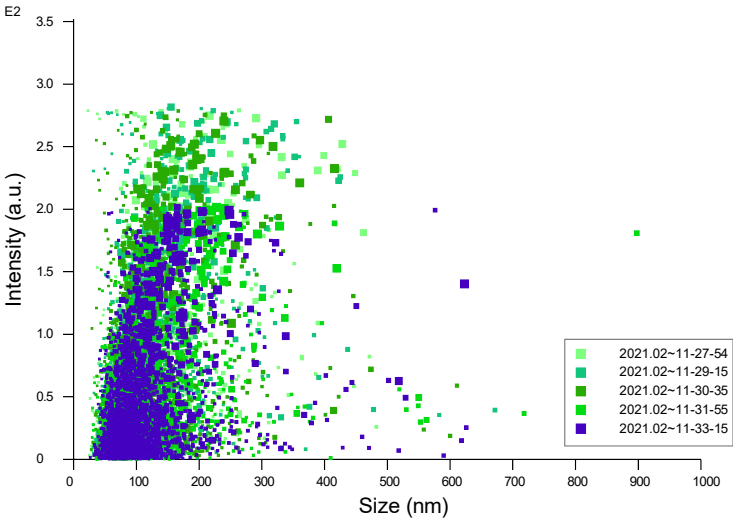
Mean:	130.9 nm
Mode:	81.7 nm
SD:	66.1 nm
D10:	77.6 nm
D50:	110.1 nm
D90:	207.8 nm

Stats: Mean +/- Standard Error

Mean:	131.3 +/- 2.1 nm
Mode:	82.4 +/- 2.2 nm
SD:	66.3 +/- 3.8 nm
D10:	77.7 +/- 0.6 nm
D50:	110.3 +/- 1.9 nm
D90:	204.2 +/- 6.2 nm
Concentration (Upgrade):	2.35e+10 +/- 1.50e+09 particles/ml
	35.6 +/- 1.7 particles/frame
	37.2 +/- 1.8 centres/frame

NANOSIGHT

2021.02.25 ECA42 2021-02-25 11-25-02



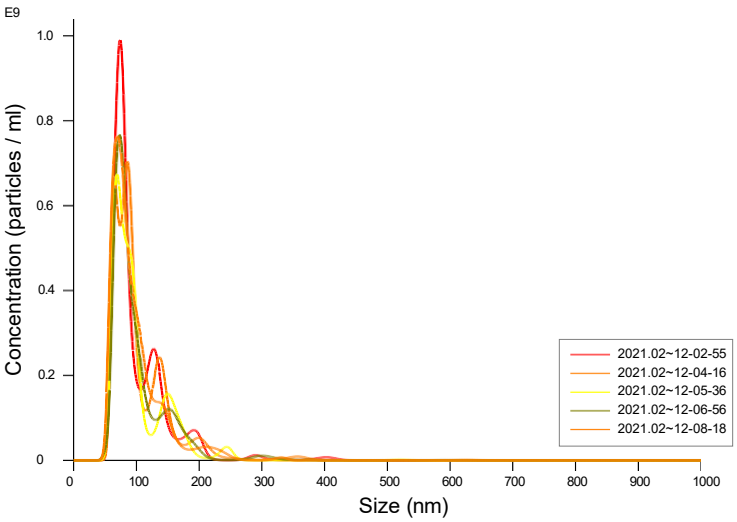
Intensity / Size graph for Experiment:
2021.02.25 ECA42 2021-02-25 11-25-02

Script Used: (Full Text):

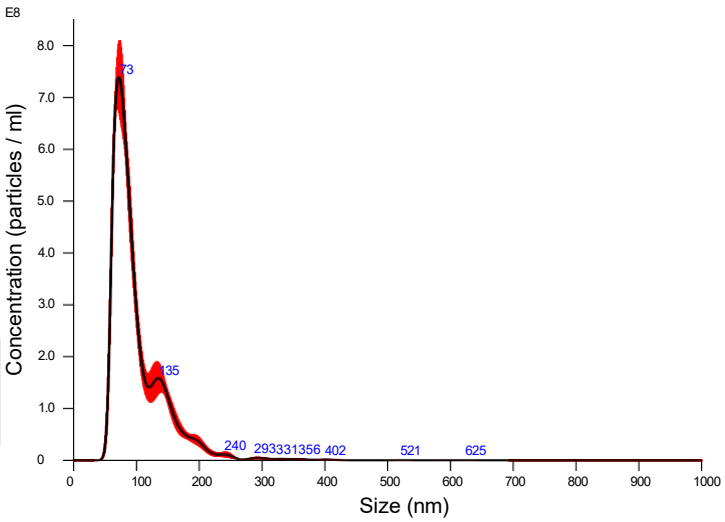
SOP Standard Measurement 11-24-12AM 25Feb2021.txt

NANOSIGHT

2021.02.25 ECA158 2021-02-25 12-00-15



FTLA Concentration / Size graph for Experiment:
2021.02.25 ECA158 2021-02-25 12-00-15



Averaged FTLA Concentration / Size for Experiment:
2021.02.25 ECA158 2021-02-25 12-00-15
Error bars indicate + / -1 standard error of the mean

Included Files

2021.02.25 ECA158 2021-02-25 12-02-55
2021.02.25 ECA158 2021-02-25 12-04-16
2021.02.25 ECA158 2021-02-25 12-05-36
2021.02.25 ECA158 2021-02-25 12-06-56
2021.02.25 ECA158 2021-02-25 12-08-18

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 11-59-50AM 25~
Time Captured:	12:00:15 25/02/2021
Operator:	Hanna
Pre-treatment:	1:50
Sample Name:	ECA158
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	15
Slider Shutter:	1206
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	24.5 - 24.7 ºC
Viscosity:	(Water) 0.894 - 0.899 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	100

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 15.5 - 16.2 pix

Results

Stats: Merged Data

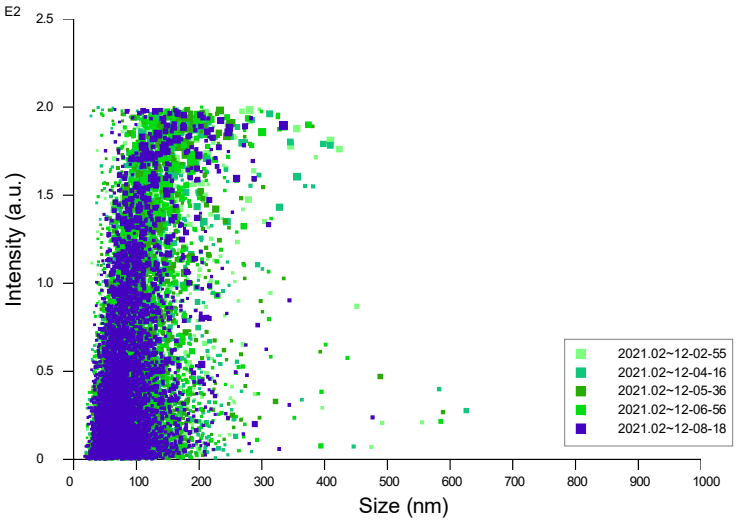
Mean:	100.7 nm
Mode:	72.0 nm
SD:	45.9 nm
D10:	63.7 nm
D50:	85.6 nm
D90:	155.9 nm

Stats: Mean +/- Standard Error

Mean:	100.8 +/- 1.2 nm
Mode:	74.4 +/- 3.0 nm
SD:	45.7 +/- 1.5 nm
D10:	63.8 +/- 0.7 nm
D50:	85.5 +/- 0.9 nm
D90:	155.3 +/- 2.2 nm
Concentration (Upgrade):	3.73e+10 +/- 1.30e+09 particles/ml
	57.4 +/- 1.9 particles/frame
	57.3 +/- 1.8 centres/frame

NANOSIGHT

2021.02.25 ECA158 2021-02-25 12-00-15



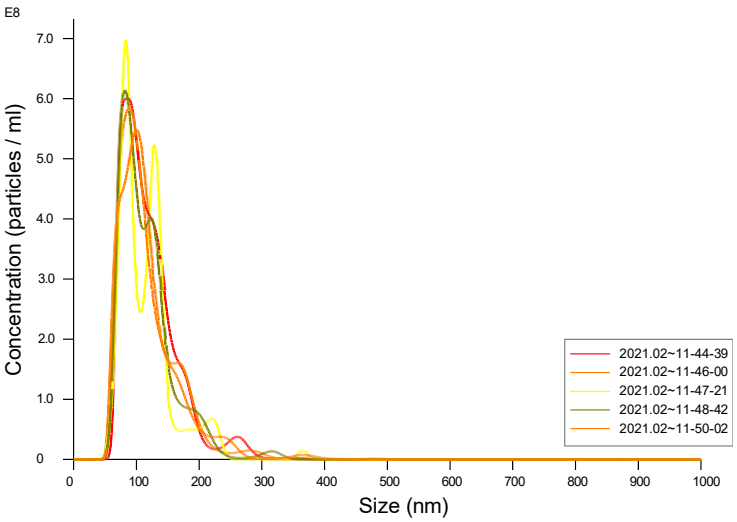
Intensity / Size graph for Experiment:
2021.02.25 ECA158 2021-02-25 12-00-15

Script Used: (Full Text):

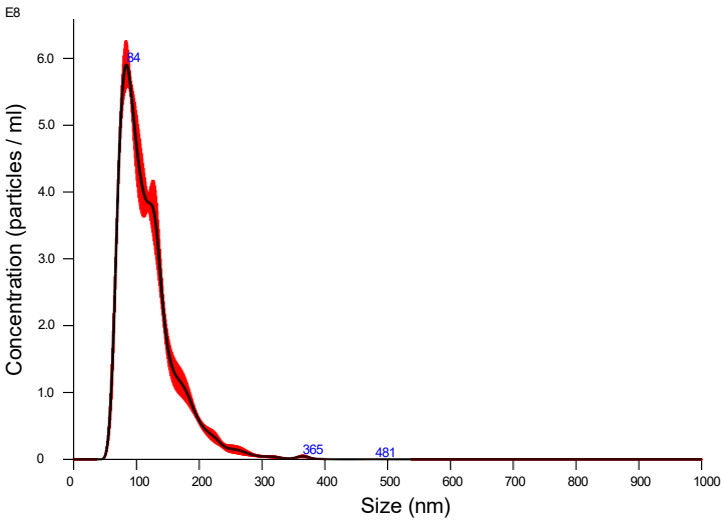
SOP Standard Measurement 11-59-50AM 25Feb2021.txt

NANOSIGHT

2021.02.25 ECV158 2021-02-25 11-41-56



FTLA Concentration / Size graph for Experiment:
2021.02.25 ECV158 2021-02-25 11-41-56



Averaged FTLA Concentration / Size for Experiment:
2021.02.25 ECV158 2021-02-25 11-41-56
Error bars indicate + / -1 standard error of the mean

Included Files

2021.02.25 ECV158 2021-02-25 11-44-39
2021.02.25 ECV158 2021-02-25 11-46-00
2021.02.25 ECV158 2021-02-25 11-47-21
2021.02.25 ECV158 2021-02-25 11-48-42
2021.02.25 ECV158 2021-02-25 11-50-02

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 11-41-50AM 25~
Time Captured:	11:41:56 25/02/2021
Operator:	Hanna
Pre-treatment:	1:50
Sample Name:	ECV158
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	15
Slider Shutter:	1206
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	23.9 - 24.2 ºC
Viscosity:	(Water) 0.906 - 0.910 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	100

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 14.0 - 15.3 pix

Results

Stats: Merged Data

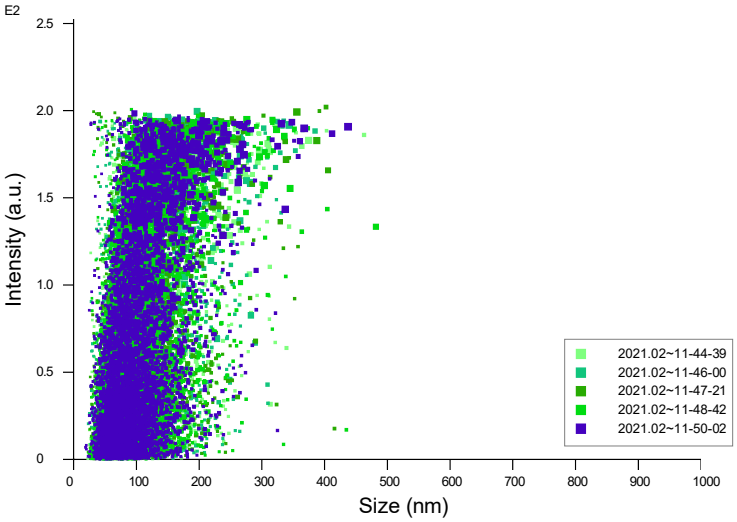
Mean:	117.5 nm
Mode:	83.9 nm
SD:	45.2 nm
D10:	73.6 nm
D50:	106.9 nm
D90:	176.2 nm

Stats: Mean +/- Standard Error

Mean:	117.4 +/- 0.8 nm
Mode:	88.0 +/- 3.3 nm
SD:	45.1 +/- 0.8 nm
D10:	73.5 +/- 0.7 nm
D50:	107.0 +/- 0.9 nm
D90:	175.4 +/- 1.3 nm
Concentration (Upgrade):	4.37e+10 +/- 1.29e+09 particles/ml
	82.3 +/- 3.0 particles/frame
	79.8 +/- 3.0 centres/frame

NANOSIGHT

2021.02.25 ECV158 2021-02-25 11-41-56



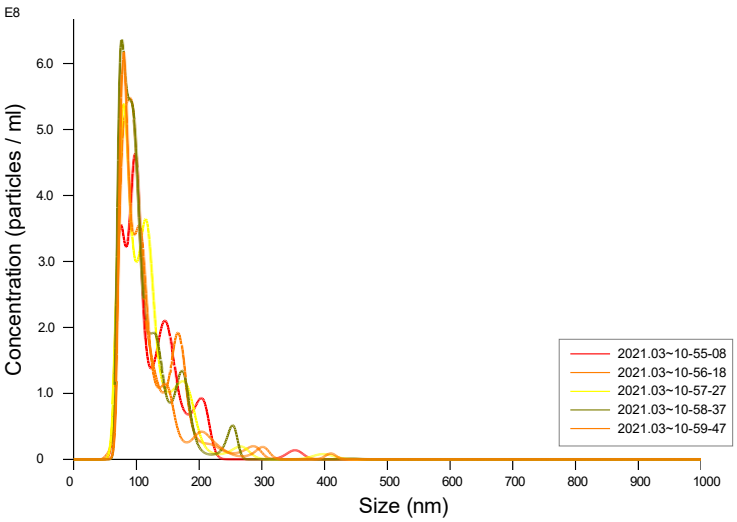
Intensity / Size graph for Experiment:
2021.02.25 ECV158 2021-02-25 11-41-56

Script Used: (Full Text):

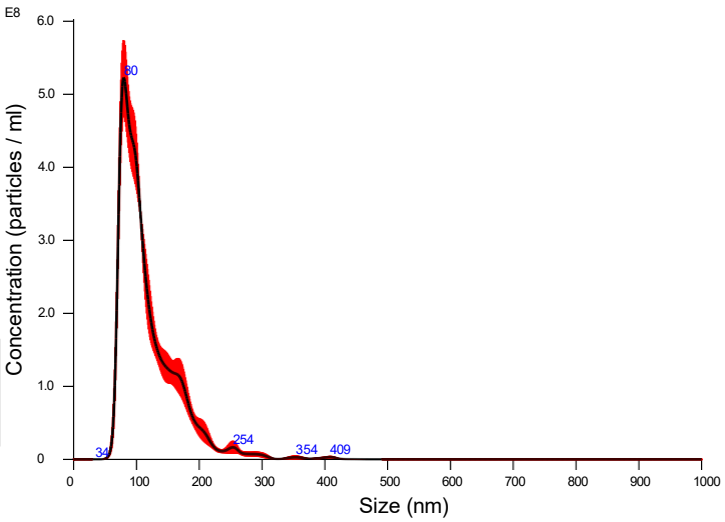
SOP Standard Measurement 11-41-50AM 25Feb2021.txt

NANOSIGHT

2021.03.12 ECA 150 2021-03-12 10-52-26



FTLA Concentration / Size graph for Experiment:
2021.03.12 ECA 150 2021-03-12 10-52-26



Averaged FTLA Concentration / Size for Experiment:
2021.03.12 ECA 150 2021-03-12 10-52-26
Error bars indicate + / -1 standard error of the mean

Included Files

2021.03.12 ECA 150 2021-03-12 10-55-08
2021.03.12 ECA 150 2021-03-12 10-56-18
2021.03.12 ECA 150 2021-03-12 10-57-27
2021.03.12 ECA 150 2021-03-12 10-58-37
2021.03.12 ECA 150 2021-03-12 10-59-47

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 10-51-38AM 12~
Time Captured:	10:52:26 12/03/2021
Operator:	Hanna
Pre-treatment:	
Sample Name:	ECA 150
Diluent:	1:50
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	23.4 - 23.6 ºC
Viscosity:	(Water) 0.918 - 0.922 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 13.3 - 13.9 pix

Results

Stats: Merged Data

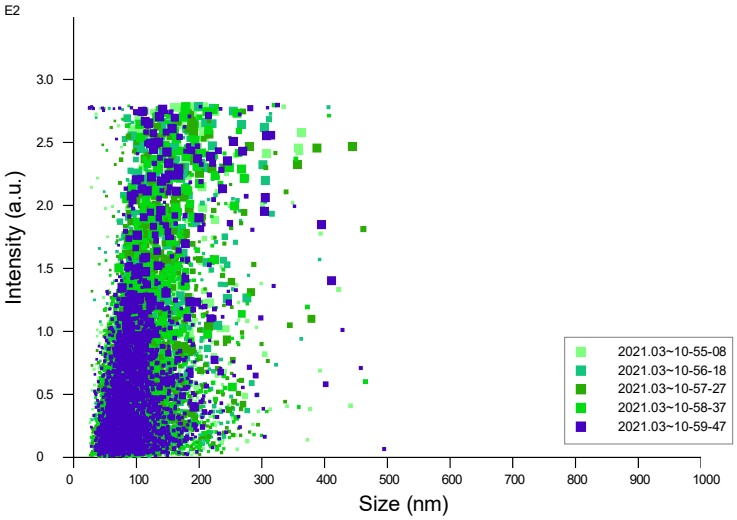
Mean:	117.9 nm
Mode:	79.8 nm
SD:	48.1 nm
D10:	75.3 nm
D50:	102.8 nm
D90:	178.3 nm

Stats: Mean +/- Standard Error

Mean:	117.9 +/- 1.5 nm
Mode:	85.1 +/- 3.9 nm
SD:	48.0 +/- 1.7 nm
D10:	75.3 +/- 0.4 nm
D50:	103.1 +/- 1.7 nm
D90:	179.6 +/- 2.5 nm
Concentration (Upgrade):	3.17e+10 +/- 1.05e+09 particles/ml
	46.3 +/- 1.5 particles/frame
	46.2 +/- 1.4 centres/frame

NANOSIGHT

2021.03.12 ECA 150 2021-03-12 10-52-26



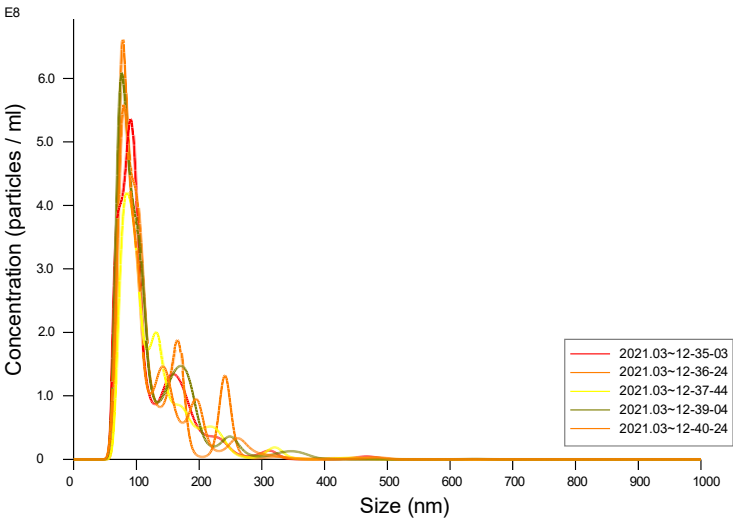
Intensity / Size graph for Experiment:
2021.03.12 ECA 150 2021-03-12 10-52-26

Script Used: (Full Text):

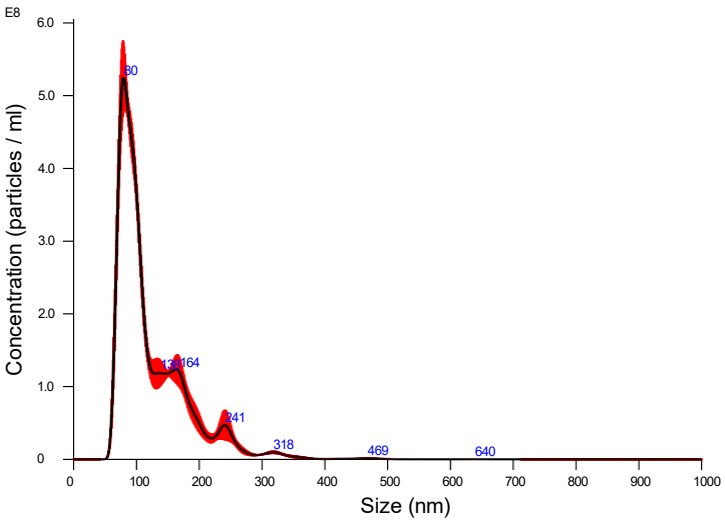
SOP Standard Measurement 10-51-38AM 12Mar2021.txt

NANOSIGHT

2021.03.25 ECA 159 2021-03-25 12-32-22



FTLA Concentration / Size graph for Experiment:
2021.03.25 ECA 159 2021-03-25 12-32-22



Averaged FTLA Concentration / Size for Experiment:
2021.03.25 ECA 159 2021-03-25 12-32-22
Error bars indicate + / -1 standard error of the mean

Included Files

2021.03.25 ECA 159 2021-03-25 12-35-03
2021.03.25 ECA 159 2021-03-25 12-36-24
2021.03.25 ECA 159 2021-03-25 12-37-44
2021.03.25 ECA 159 2021-03-25 12-39-04
2021.03.25 ECA 159 2021-03-25 12-40-24

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 12-31-05PM 25~
Time Captured:	12:32:22 25/03/2021
Operator:	Hanna
Pre-treatment:	1:50
Sample Name:	ECA 159
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	23.7 - 23.9 ºC
Viscosity:	(Water) 0.912 - 0.916 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 12.9 - 13.9 pix

Results

Stats: Merged Data

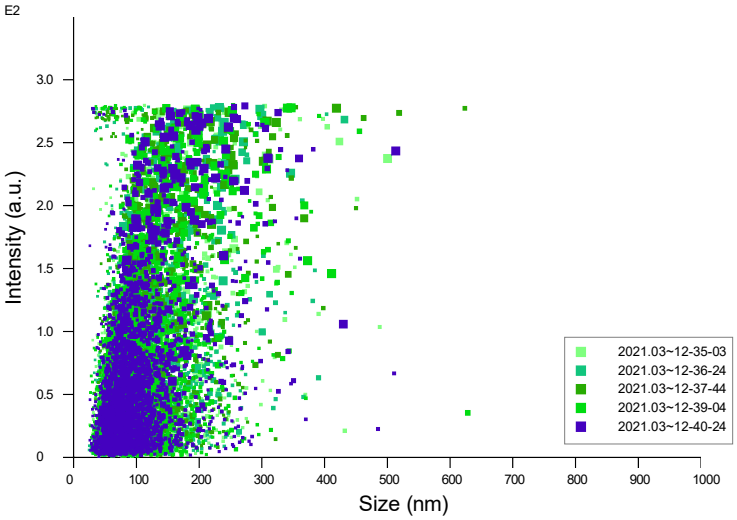
Mean:	122.7 nm
Mode:	79.5 nm
SD:	58.8 nm
D10:	73.1 nm
D50:	100.6 nm
D90:	198.5 nm

Stats: Mean +/- Standard Error

Mean:	122.8 +/- 1.4 nm
Mode:	82.1 +/- 2.6 nm
SD:	58.7 +/- 1.0 nm
D10:	73.3 +/- 1.2 nm
D50:	101.1 +/- 2.0 nm
D90:	203.5 +/- 6.3 nm
Concentration (Upgrade):	3.19e+10 +/- 1.25e+09 particles/ml
	47.7 +/- 1.4 particles/frame
	49.0 +/- 1.1 centres/frame

NANOSIGHT

2021.03.25 ECA 159 2021-03-25 12-32-22



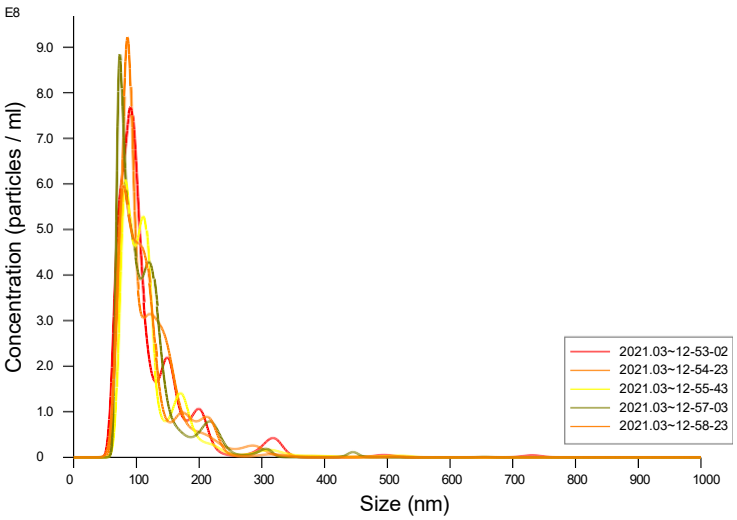
Intensity / Size graph for Experiment:
2021.03.25 ECA 159 2021-03-25 12-32-22

Script Used: (Full Text):

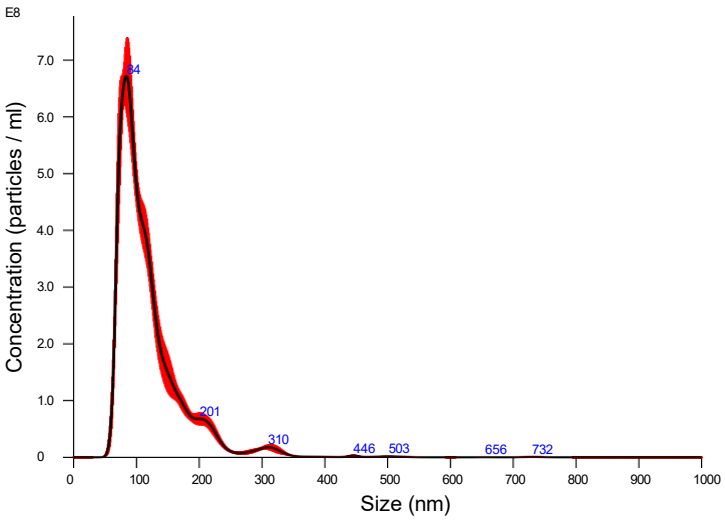
SOP Standard Measurement 12-31-05PM 25Mar2021.txt

NANOSIGHT

2021.03.25 ECV42 2021-03-25 12-50-21



FTLA Concentration / Size graph for Experiment:
2021.03.25 ECV42 2021-03-25 12-50-21



Averaged FTLA Concentration / Size for Experiment:
2021.03.25 ECV42 2021-03-25 12-50-21
Error bars indicate + / -1 standard error of the mean

Included Files

2021.03.25 ECV42 2021-03-25 12-53-02
2021.03.25 ECV42 2021-03-25 12-54-23
2021.03.25 ECV42 2021-03-25 12-55-43
2021.03.25 ECV42 2021-03-25 12-57-03
2021.03.25 ECV42 2021-03-25 12-58-23

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 12-31-05PM 25~
Time Captured:	12:50:21 25/03/2021
Operator:	Hanna
Pre-treatment:	1:50
Sample Name:	ECV 42
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	24.2 - 24.4 ºC
Viscosity:	(Water) 0.901 - 0.905 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 13.3 - 15.1 pix

Results

Stats: Merged Data

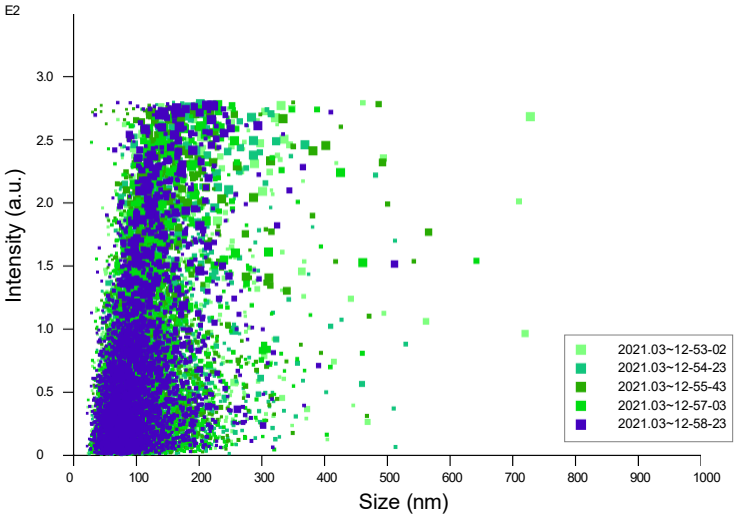
Mean:	119.6 nm
Mode:	83.8 nm
SD:	57.7 nm
D10:	73.5 nm
D50:	103.0 nm
D90:	186.9 nm

Stats: Mean +/- Standard Error

Mean:	119.6 +/- 1.3 nm
Mode:	82.2 +/- 2.9 nm
SD:	57.0 +/- 3.7 nm
D10:	73.8 +/- 1.3 nm
D50:	103.1 +/- 1.3 nm
D90:	186.4 +/- 2.5 nm
Concentration (Upgrade):	4.40e+10 +/- 1.92e+09 particles/ml
	64.7 +/- 2.4 particles/frame
	63.7 +/- 2.1 centres/frame

NANOSIGHT

2021.03.25 ECV42 2021-03-25 12-50-21



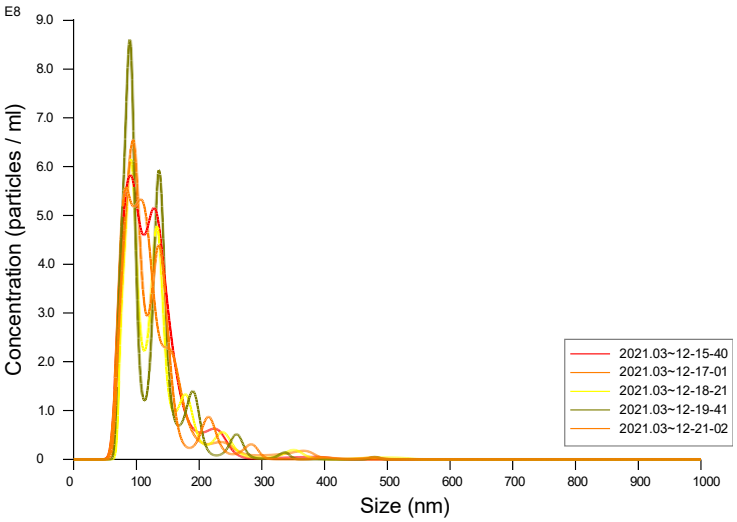
Intensity / Size graph for Experiment:
2021.03.25 ECV42 2021-03-25 12-50-21

Script Used: (Full Text):

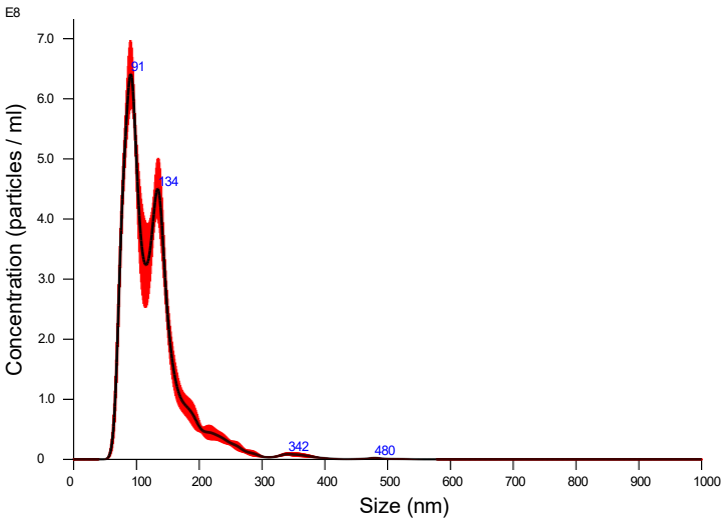
SOP Standard Measurement 12-31-05PM 25Mar2021.txt

NANOSIGHT

2021.03.25 ECV150 2021-03-25 12-12-42



FTLA Concentration / Size graph for Experiment:
2021.03.25 ECV150 2021-03-25 12-12-42



Averaged FTLA Concentration / Size for Experiment:
2021.03.25 ECV150 2021-03-25 12-12-42
Error bars indicate + / -1 standard error of the mean

Included Files

2021.03.25 ECV150 2021-03-25 12-15-40
2021.03.25 ECV150 2021-03-25 12-17-01
2021.03.25 ECV150 2021-03-25 12-18-21
2021.03.25 ECV150 2021-03-25 12-19-41
2021.03.25 ECV150 2021-03-25 12-21-02

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 12-11-00PM 25~
Time Captured:	12:12:42 25/03/2021
Operator:	Hanna
Pre-treatment:	1:50
Sample Name:	ECV 150
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	23.1 - 23.3 ºC
Viscosity:	(Water) 0.925 - 0.928 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 12.7 - 13.5 pix

Results

Stats: Merged Data

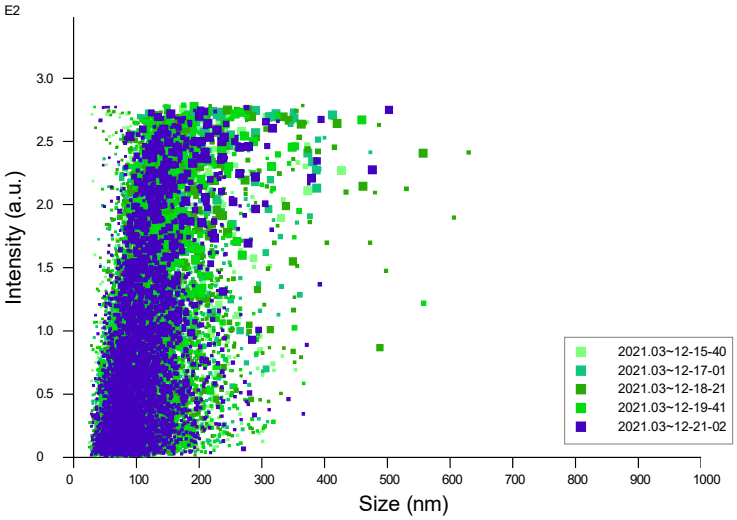
Mean:	126.5 nm
Mode:	90.9 nm
SD:	52.7 nm
D10:	79.6 nm
D50:	114.9 nm
D90:	186.3 nm

Stats: Mean +/- Standard Error

Mean:	126.7 +/- 1.9 nm
Mode:	90.0 +/- 1.8 nm
SD:	52.7 +/- 2.8 nm
D10:	79.8 +/- 0.9 nm
D50:	115.0 +/- 2.0 nm
D90:	186.4 +/- 3.8 nm
Concentration (Upgrade):	4.38e+10 +/- 1.91e+09 particles/ml
	71.5 +/- 3.2 particles/frame
	71.5 +/- 2.7 centres/frame

NANOSIGHT

2021.03.25 ECV150 2021-03-25 12-12-42



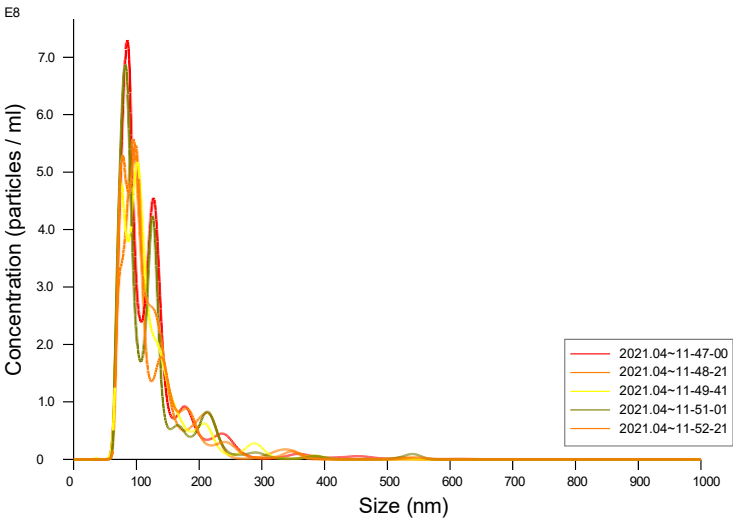
Intensity / Size graph for Experiment:
2021.03.25 ECV150 2021-03-25 12-12-42

Script Used: (Full Text):

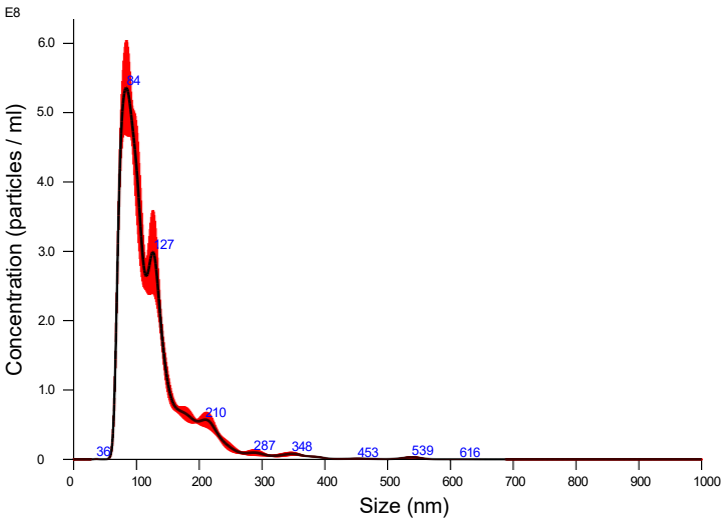
SOP Standard Measurement 12-11-00PM 25Mar2021.txt

NANOSIGHT

2021.04.14 ECA 1PE 2021-04-14 11-44-07



FTLA Concentration / Size graph for Experiment:
2021.04.14 ECA 1PE 2021-04-14 11-44-07



Averaged FTLA Concentration / Size for Experiment:
2021.04.14 ECA 1PE 2021-04-14 11-44-07
Error bars indicate + / -1 standard error of the mean

Included Files

2021.04.14 ECA 1PE 2021-04-14 11-47-00
2021.04.14 ECA 1PE 2021-04-14 11-48-21
2021.04.14 ECA 1PE 2021-04-14 11-49-41
2021.04.14 ECA 1PE 2021-04-14 11-51-01
2021.04.14 ECA 1PE 2021-04-14 11-52-21

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 11-44-00AM 14A~
Time Captured:	11:44:07 14/04/2021
Operator:	Hanna
Pre-treatment:	1:50
Sample Name:	ECA 1PE
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	23.0 - 23.2 ºC
Viscosity:	(Water) 0.927 - 0.931 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 13.2 - 13.5 pix

Results

Stats: Merged Data

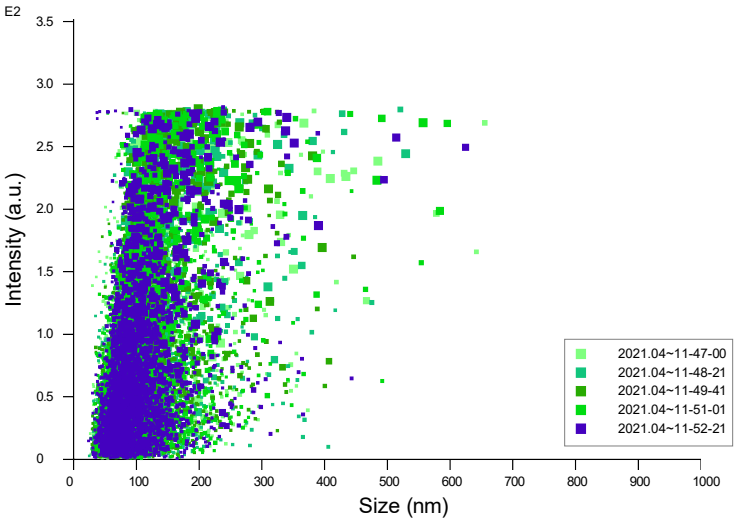
Mean:	123.5 nm
Mode:	83.8 nm
SD:	59.8 nm
D10:	76.0 nm
D50:	105.5 nm
D90:	196.2 nm

Stats: Mean +/- Standard Error

Mean:	123.5 +/- 0.8 nm
Mode:	92.8 +/- 3.7 nm
SD:	59.5 +/- 2.1 nm
D10:	76.1 +/- 0.5 nm
D50:	105.6 +/- 1.1 nm
D90:	196.0 +/- 3.4 nm
Concentration (Upgrade):	3.54e+10 +/- 1.12e+09 particles/ml
	57.6 +/- 1.9 particles/frame
	56.4 +/- 1.6 centres/frame

NANOSIGHT

2021.04.14 ECA 1PE 2021-04-14 11-44-07



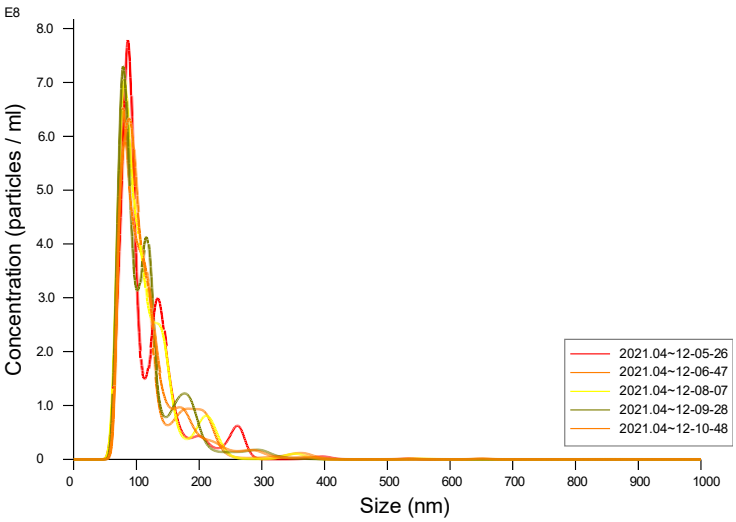
Intensity / Size graph for Experiment:
2021.04.14 ECA 1PE 2021-04-14 11-44-07

Script Used: (Full Text):

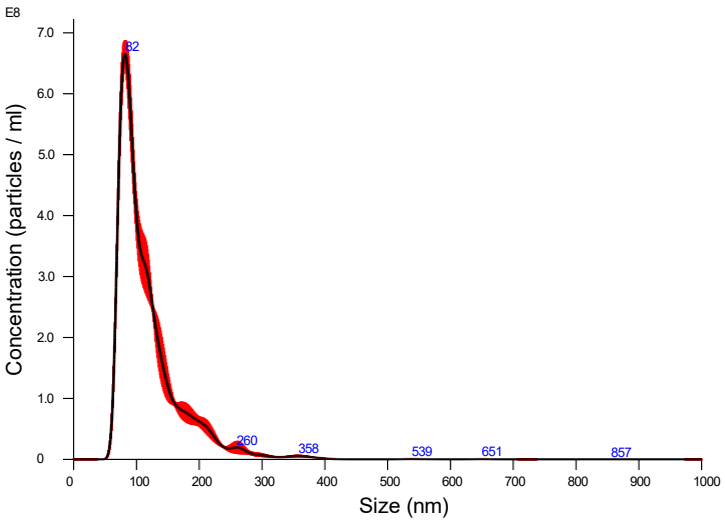
SOP Standard Measurement 11-44-00AM 14Apr2021.txt

NANOSIGHT

2021.04.14 ECA 18PE 2021-04-14 12-02-40



FTLA Concentration / Size graph for Experiment:
2021.04.14 ECA 18PE 2021-04-14 12-02-40



Averaged FTLA Concentration / Size for Experiment:
2021.04.14 ECA 18PE 2021-04-14 12-02-40
Error bars indicate + / -1 standard error of the mean

Included Files

2021.04.14 ECA 18PE 2021-04-14 12-05-26
2021.04.14 ECA 18PE 2021-04-14 12-06-47
2021.04.14 ECA 18PE 2021-04-14 12-08-07
2021.04.14 ECA 18PE 2021-04-14 12-09-28
2021.04.14 ECA 18PE 2021-04-14 12-10-48

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 12-02-07PM 14A~
Time Captured:	12:02:40 14/04/2021
Operator:	Hanna
Pre-treatment:	1:50
Sample Name:	ECA 1PE
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	23.6 - 23.8 ºC
Viscosity:	(Water) 0.913 - 0.918 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 13.5 - 14.2 pix

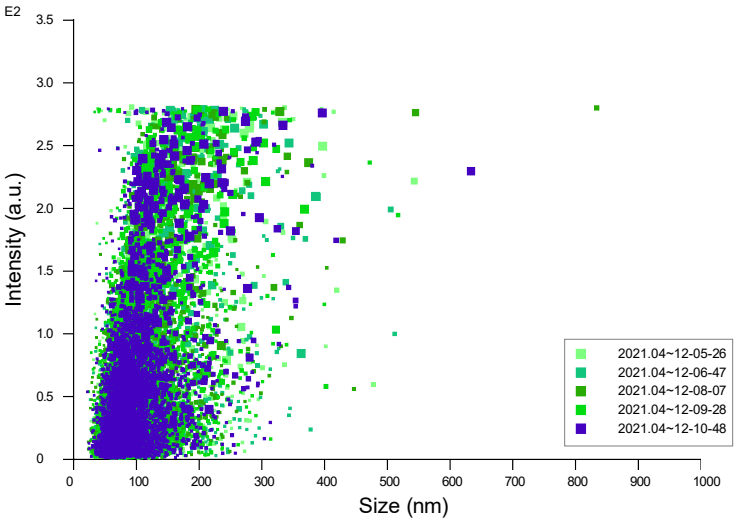
Results

Stats: Merged Data

Mean:	117.8 nm
Mode:	81.6 nm
SD:	53.6 nm
D10:	73.7 nm
D50:	100.9 nm
D90:	187.1 nm

Stats: Mean +/- Standard Error

Mean:	117.9 +/- 0.8 nm
Mode:	82.5 +/- 2.1 nm
SD:	53.5 +/- 0.9 nm
D10:	73.8 +/- 0.5 nm
D50:	100.8 +/- 0.7 nm
D90:	186.5 +/- 3.0 nm
Concentration (Upgrade):	3.86e+10 +/- 8.66e+08 particles/ml
	55.6 +/- 1.1 particles/frame
	55.0 +/- 1.1 centres/frame



Intensity / Size graph for Experiment:
2021.04.14 ECA 18PE 2021-04-14 12-02-40

Script Used: (Full Text):

SOP Standard Measurement 12-02-07PM 14Apr2021.txt

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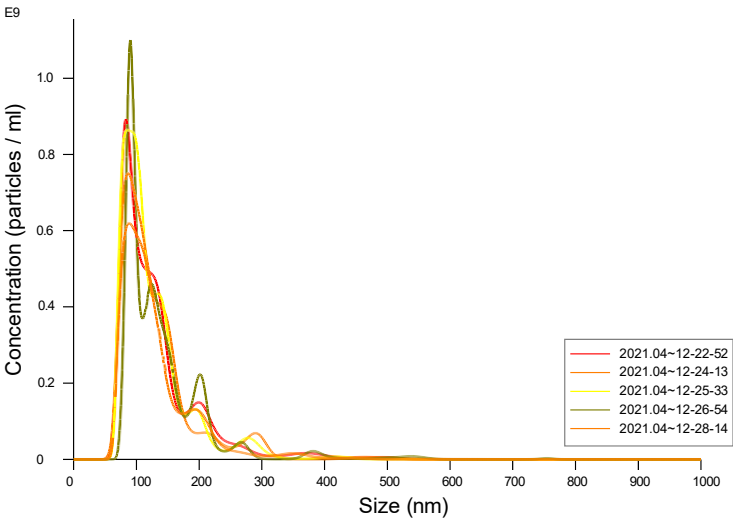
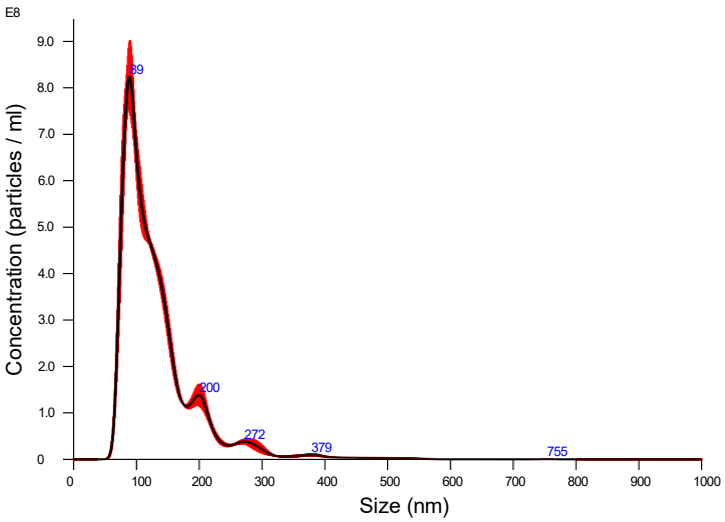
2021.04.14 ECV 159 2021-04-14 12-20-08



FTLA Concentration / Size graph for Experiment:
2021.04.14 ECV 159 2021-04-14 12-20-08



Averaged FTLA Concentration / Size for Experiment:
2021.04.14 ECV 159 2021-04-14 12-20-08
Error bars indicate + / -1 standard error of the mean

Included Files

2021.04.14 ECV 159 2021-04-14 12-22-52
2021.04.14 ECV 159 2021-04-14 12-24-13
2021.04.14 ECV 159 2021-04-14 12-25-33
2021.04.14 ECV 159 2021-04-14 12-26-54
2021.04.14 ECV 159 2021-04-14 12-28-14

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 12-19-44PM 14A~
Time Captured:	12:20:08 14/04/2021
Operator:	Hanna
Pre-treatment:	1:50
Sample Name:	ECV 159
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	24.2 - 24.4 ºC
Viscosity:	(Water) 0.901 - 0.905 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 12.1 - 13.4 pix

Results

Stats: Merged Data

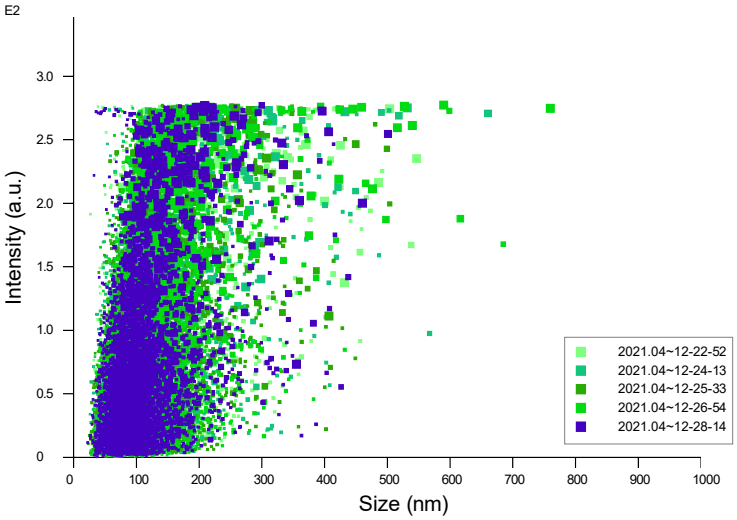
Mean:	131.7 nm
Mode:	89.0 nm
SD:	63.6 nm
D10:	79.5 nm
D50:	113.7 nm
D90:	204.8 nm

Stats: Mean +/- Standard Error

Mean:	131.9 +/- 2.3 nm
Mode:	86.8 +/- 1.2 nm
SD:	63.2 +/- 3.1 nm
D10:	79.7 +/- 1.6 nm
D50:	114.1 +/- 1.9 nm
D90:	204.6 +/- 2.9 nm
Concentration (Upgrade):	5.83e+10 +/- 2.41e+09 particles/ml
	95.3 +/- 3.8 particles/frame
	90.9 +/- 3.2 centres/frame

NANOSIGHT

2021.04.14 ECV 159 2021-04-14 12-20-08



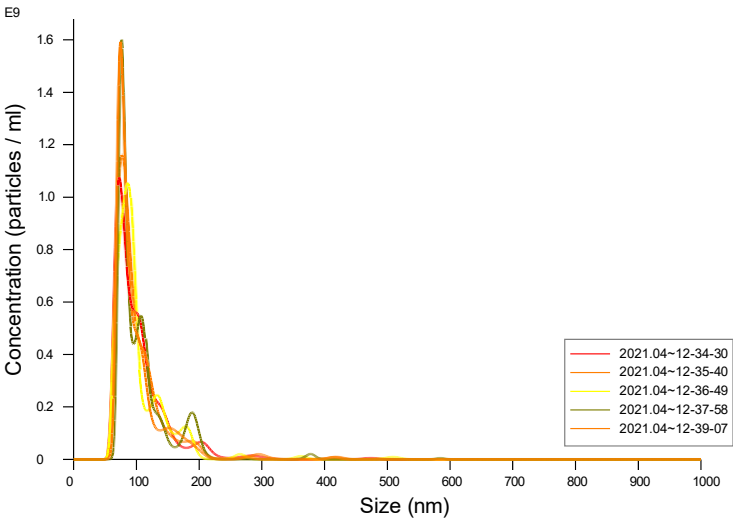
Intensity / Size graph for Experiment:
2021.04.14 ECV 159 2021-04-14 12-20-08

Script Used: (Full Text):

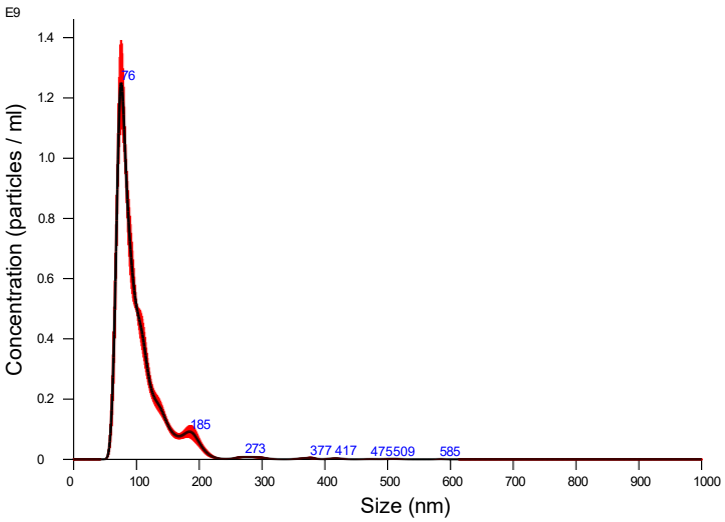
SOP Standard Measurement 12-19-44PM 14Apr2021.txt

NANOSIGHT

2021.04.27 ECA 10PE 2021-04-27 12-31-49



FTLA Concentration / Size graph for Experiment:
2021.04.27 ECA 10PE 2021-04-27 12-31-49



Averaged FTLA Concentration / Size for Experiment:
2021.04.27 ECA 10PE 2021-04-27 12-31-49
Error bars indicate + / -1 standard error of the mean

Included Files

2021.04.27 ECA 10PE 2021-04-27 12-34-30
2021.04.27 ECA 10PE 2021-04-27 12-35-40
2021.04.27 ECA 10PE 2021-04-27 12-36-49
2021.04.27 ECA 10PE 2021-04-27 12-37-58
2021.04.27 ECA 10PE 2021-04-27 12-39-07

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 12-31-26PM 27A~
Time Captured:	12:31:49 27/04/2021
Operator:	Hanna
Pre-treatment:	1:5
Sample Name:	ECA 10PE
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	25.2 - 25.3 ºC
Viscosity:	(Water) 0.882 - 0.885 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 14.0 - 15.1 pix

Results

Stats: Merged Data

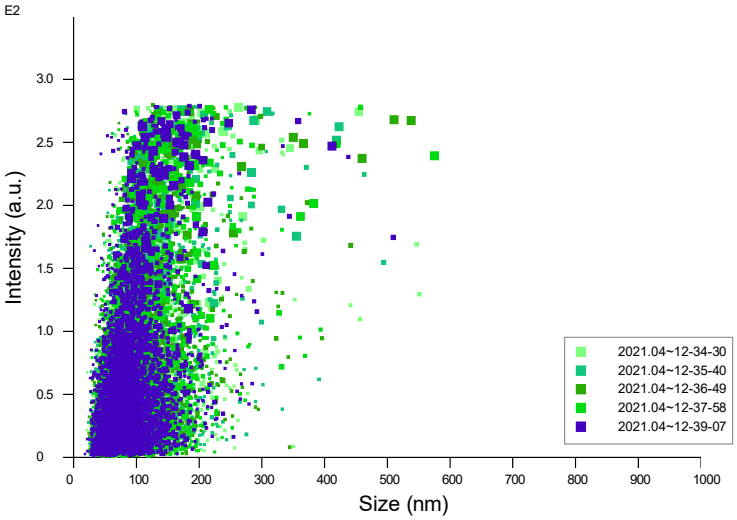
Mean:	103.6 nm
Mode:	75.6 nm
SD:	45.4 nm
D10:	69.9 nm
D50:	89.4 nm
D90:	154.9 nm

Stats: Mean +/- Standard Error

Mean:	103.6 +/- 1.3 nm
Mode:	77.3 +/- 2.4 nm
SD:	45.2 +/- 1.8 nm
D10:	69.8 +/- 0.8 nm
D50:	89.5 +/- 1.2 nm
D90:	156.9 +/- 4.6 nm
Concentration (Upgrade):	5.09e+10 +/- 5.35e+08 particles/ml
	70.9 +/- 0.7 particles/frame
	69.4 +/- 0.6 centres/frame

NANOSIGHT

2021.04.27 ECA 10PE 2021-04-27 12-31-49



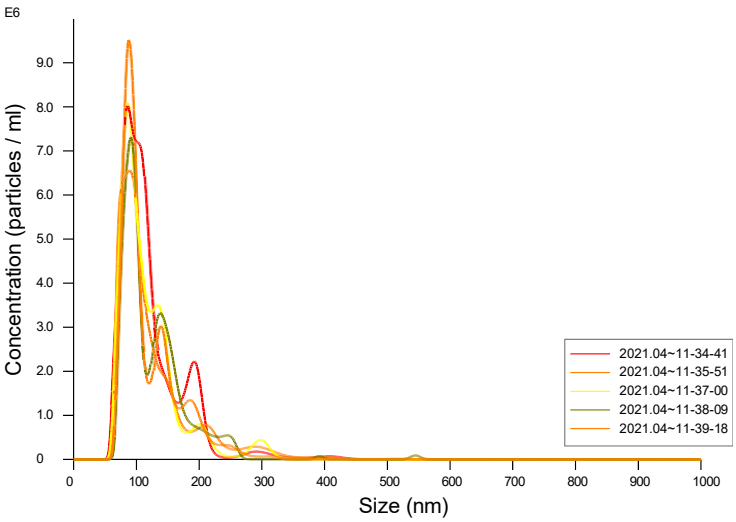
Intensity / Size graph for Experiment:
2021.04.27 ECA 10PE 2021-04-27 12-31-49

Script Used: (Full Text):

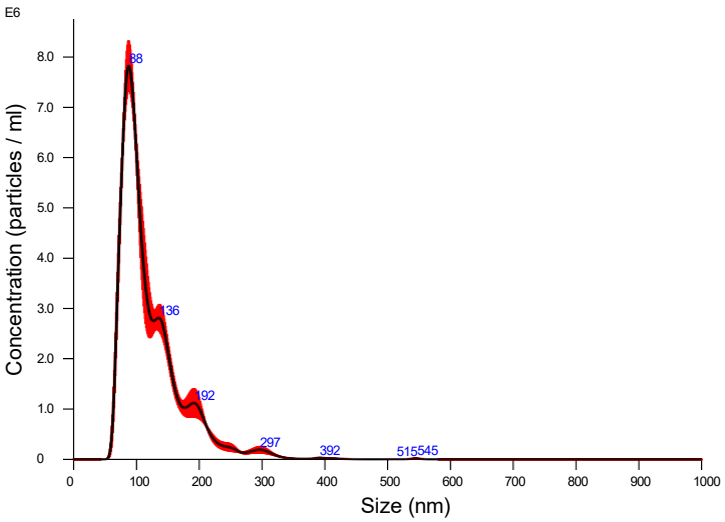
SOP Standard Measurement 12-31-26PM 27Apr2021.txt

NANOSIGHT

2021.04.27 ECV 6PE 2021-04-27 11-32-02



FTLA Concentration / Size graph for Experiment:
2021.04.27 ECV 6PE 2021-04-27 11-32-02



Averaged FTLA Concentration / Size for Experiment:
2021.04.27 ECV 6PE 2021-04-27 11-32-02
Error bars indicate + / -1 standard error of the mean

Included Files

2021.04.27 ECV 6PE 2021-04-27 11-34-41
2021.04.27 ECV 6PE 2021-04-27 11-35-51
2021.04.27 ECV 6PE 2021-04-27 11-37-00
2021.04.27 ECV 6PE 2021-04-27 11-38-09
2021.04.27 ECV 6PE 2021-04-27 11-39-18

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 11-31-36AM 27A~
Time Captured:	11:32:02 27/04/2021
Operator:	Hanna
Pre-treatment:	1:50
Sample Name:	ECV 6PE
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	24.7 - 24.9 ºC
Viscosity:	(Water) 0.890 - 0.894 cP
Dilution factor:	Dilution not recorded
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 13.1 - 14.2 pix

Results

Stats: Merged Data

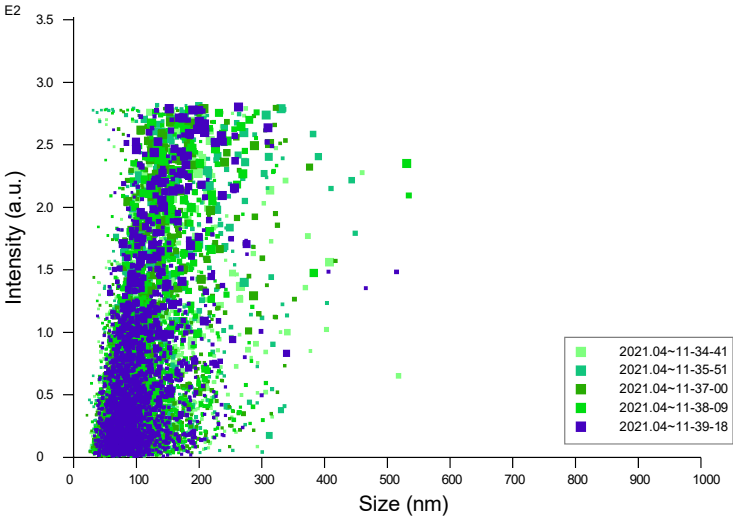
Mean:	121.9 nm
Mode:	87.7 nm
SD:	52.2 nm
D10:	76.6 nm
D50:	104.5 nm
D90:	190.8 nm

Stats: Mean +/- Standard Error

Mean:	122.0 +/- 1.3 nm
Mode:	88.1 +/- 1.0 nm
SD:	52.1 +/- 1.9 nm
D10:	76.7 +/- 0.9 nm
D50:	104.7 +/- 1.4 nm
D90:	191.4 +/- 4.4 nm
Concentration (Upgrade):	4.81e+08 +/- 2.29e+07 particles/ml
	36.0 +/- 1.4 particles/frame
	36.5 +/- 1.3 centres/frame

NANOSIGHT

2021.04.27 ECV 6PE 2021-04-27 11-32-02



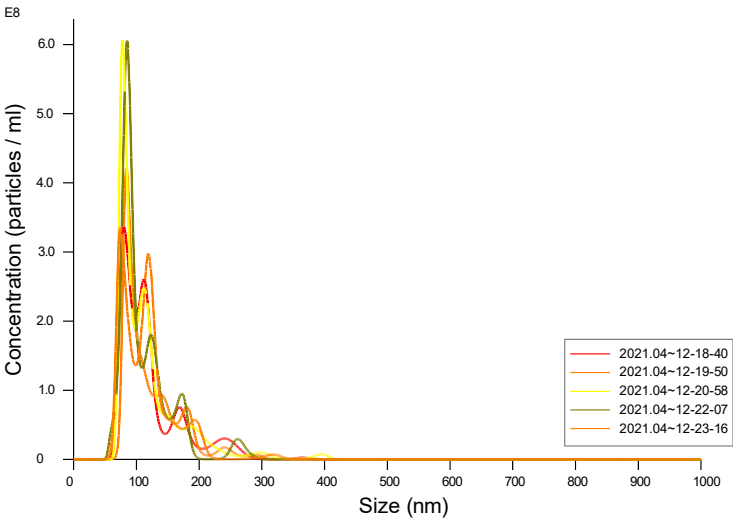
Intensity / Size graph for Experiment:
2021.04.27 ECV 6PE 2021-04-27 11-32-02

Script Used: (Full Text):

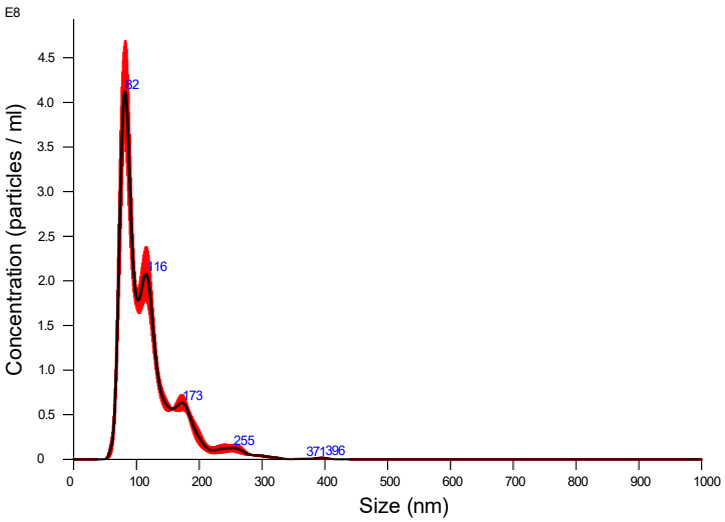
SOP Standard Measurement 11-31-36AM 27Apr2021.txt

NANOSIGHT

2021.04.27 ECV 10-2PE 2021-04-27 12-15-52



FTLA Concentration / Size graph for Experiment:
2021.04.27 ECV 10-2PE 2021-04-27 12-15-52



Averaged FTLA Concentration / Size for Experiment:
2021.04.27 ECV 10-2PE 2021-04-27 12-15-52
Error bars indicate + / -1 standard error of the mean

Included Files

2021.04.27 ECV 10-2PE 2021-04-27 12-18-40
2021.04.27 ECV 10-2PE 2021-04-27 12-19-50
2021.04.27 ECV 10-2PE 2021-04-27 12-20-58
2021.04.27 ECV 10-2PE 2021-04-27 12-22-07
2021.04.27 ECV 10-2PE 2021-04-27 12-23-16

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 12-15-28PM 27A~
Time Captured:	12:15:52 27/04/2021
Operator:	Hanna
Pre-treatment:	1:5
Sample Name:	ECV 10-2PE
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14 - 15
Slider Shutter:	1206 - 1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	25.5 - 25.6 ºC
Viscosity:	(Water) 0.877 - 0.880 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 12.9 - 14.2 pix

Results

Stats: Merged Data

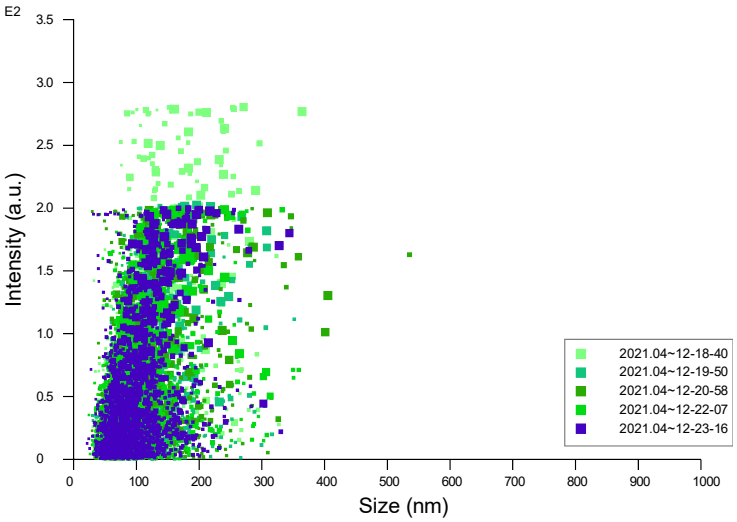
Mean:	116.5 nm
Mode:	81.8 nm
SD:	47.1 nm
D10:	75.5 nm
D50:	102.9 nm
D90:	178.5 nm

Stats: Mean +/- Standard Error

Mean:	116.8 +/- 1.6 nm
Mode:	80.2 +/- 2.0 nm
SD:	46.6 +/- 2.1 nm
D10:	75.8 +/- 1.4 nm
D50:	103.2 +/- 2.7 nm
D90:	180.1 +/- 3.5 nm
Concentration (Upgrade):	2.05e+10 +/- 1.16e+09 particles/ml
	33.8 +/- 2.0 particles/frame
	34.4 +/- 1.8 centres/frame

NANOSIGHT

2021.04.27 ECV 10-2PE 2021-04-27 12-15-52



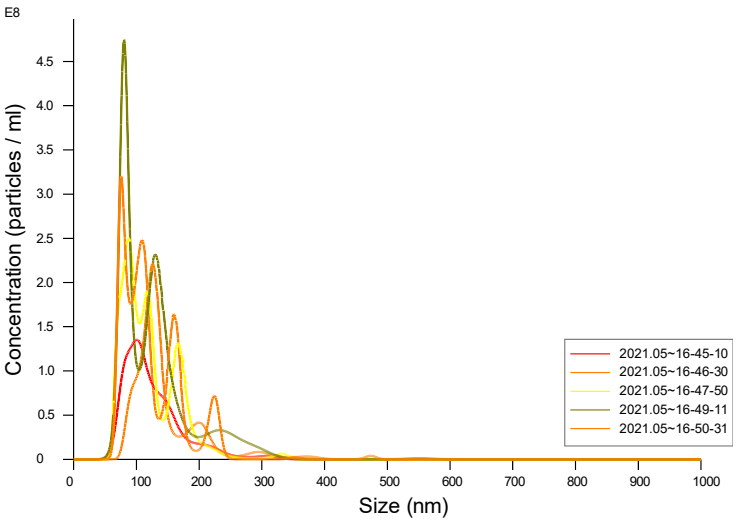
Intensity / Size graph for Experiment:
2021.04.27 ECV 10-2PE 2021-04-27 12-15-52

Script Used: (Full Text):

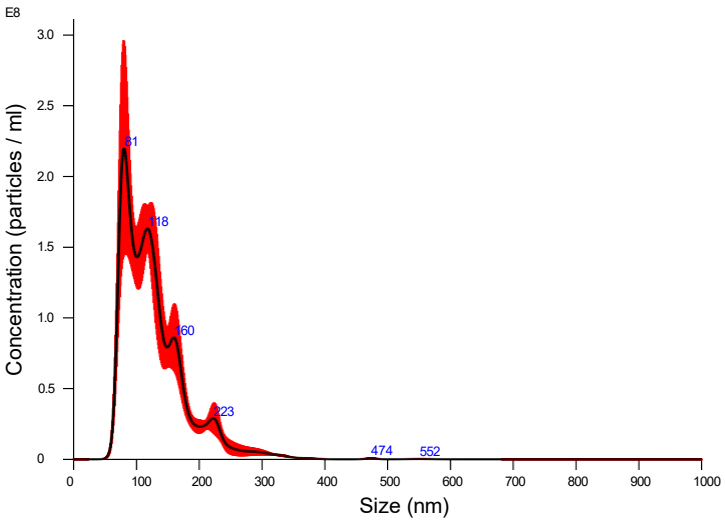
SOP Standard Measurement 12-15-28PM 27Apr2021.txt

NANOSIGHT

2021.05.06 ECV 14PE neuverd nnt 2021-05-06 16-42-18



FTLA Concentration / Size graph for Experiment:
2021.05.06 ECV 14PE neuverd nnt 2021-05-06 16-42-18



Averaged FTLA Concentration / Size for Experiment:
2021.05.06 ECV 14PE neuverd nnt 2021-05-06 16-42-18
Error bars indicate + / -1 standard error of the mean

Included Files

2021.05.06 ECV 14PE neuverd nnt 2021-05-06 16-45-10
2021.05.06 ECV 14PE neuverd nnt 2021-05-06 16-46-30
2021.05.06 ECV 14PE neuverd nnt 2021-05-06 16-47-50
2021.05.06 ECV 14PE neuverd nnt 2021-05-06 16-49-11
2021.05.06 ECV 14PE neuverd nnt 2021-05-06 16-50-31

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 04-41-06PM 06~
Time Captured:	16:42:18 06/05/2021
Operator:	Waltraud
Pre-treatment:	1:40
Sample Name:	ECV 14PE neuverd nnt
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	15
Slider Shutter:	1206
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	23.0 - 23.2 ºC
Viscosity:	(Water) 0.927 - 0.931 cP
Dilution factor:	4 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 10.7 - 14.2 pix

Results

Stats: Merged Data

Mean:	127.6 nm
Mode:	80.1 nm
SD:	53.4 nm
D10:	76.5 nm
D50:	115.9 nm
D90:	195.9 nm

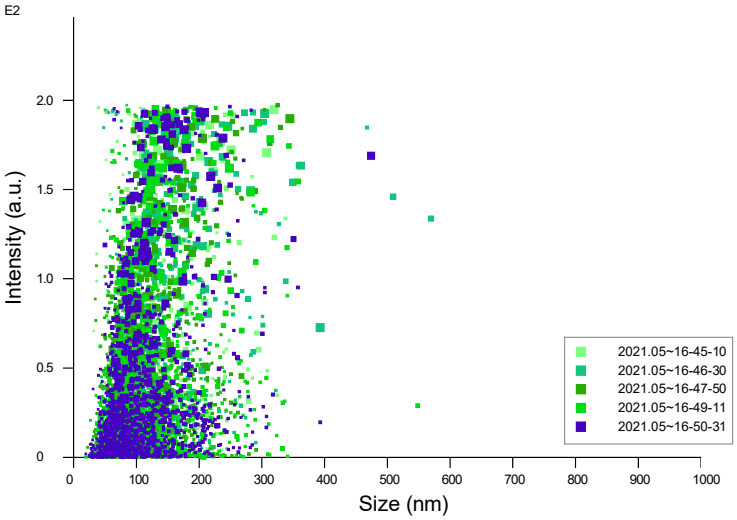
Stats: Mean +/- Standard Error

Mean:	128.7 +/- 4.5 nm
Mode:	94.1 +/- 9.0 nm
SD:	52.3 +/- 2.8 nm
D10:	80.0 +/- 4.3 nm
D50:	115.8 +/- 3.4 nm
D90:	195.7 +/- 7.6 nm
Concentration (Upgrade):	1.68e+10 +/- 2.38e+09 particles/ml
	32.1 +/- 3.7 particles/frame
	50.8 +/- 10.4 centres/frame

Concentration measurements may be unreliable
See summary file for more info

NANOSIGHT

2021.05.06 ECV 14PE neuverd nnt 2021-05-06 16-42-18



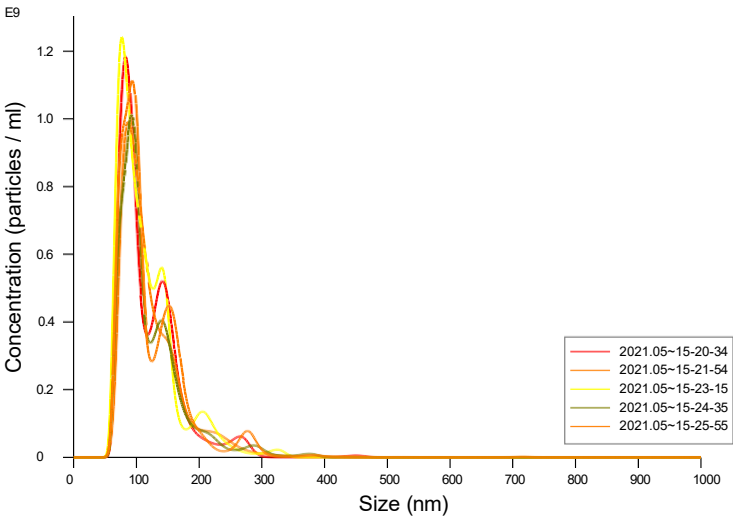
Intensity / Size graph for Experiment:
2021.05.06 ECV 14PE neuverd nnt 2021-05-06 16-42-18

Script Used: (Full Text):

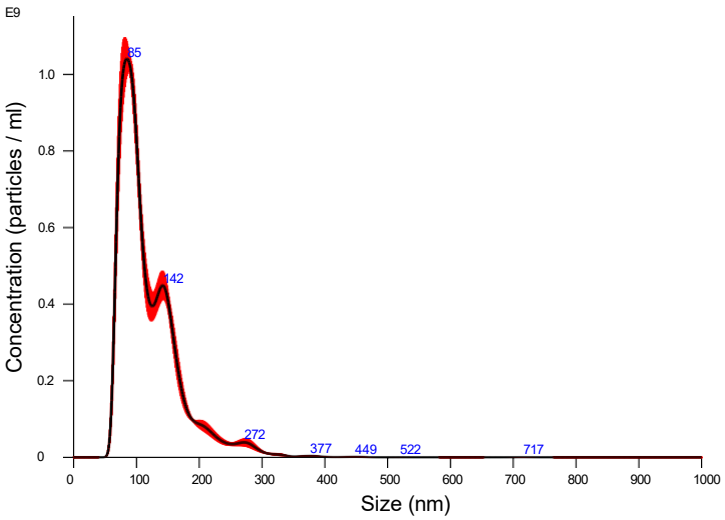
SOP Standard Measurement 04-41-06PM 06May2021.txt

NANOSIGHT

2021.05.06 ECV 17PE 2021-05-06 15-17-30



FTLA Concentration / Size graph for Experiment:
2021.05.06 ECV 17PE 2021-05-06 15-17-30



Averaged FTLA Concentration / Size for Experiment:
2021.05.06 ECV 17PE 2021-05-06 15-17-30
Error bars indicate + / -1 standard error of the mean

Included Files

2021.05.06 ECV 17PE 2021-05-06 15-20-34
2021.05.06 ECV 17PE 2021-05-06 15-21-54
2021.05.06 ECV 17PE 2021-05-06 15-23-15
2021.05.06 ECV 17PE 2021-05-06 15-24-35
2021.05.06 ECV 17PE 2021-05-06 15-25-55

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 03-13-28PM 06~
Time Captured:	15:17:30 06/05/2021
Operator:	Waltraud
Pre-treatment:	1:50
Sample Name:	ECV 17PE
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	21.6 - 21.9 ºC
Viscosity:	(Water) 0.955 - 0.961 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 13.1 - 14.2 pix

Results

Stats: Merged Data

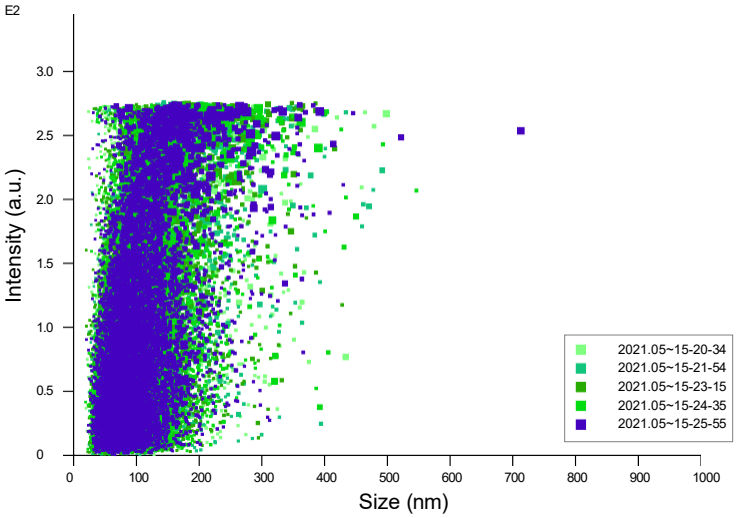
Mean:	119.4 nm
Mode:	84.8 nm
SD:	50.4 nm
D10:	73.5 nm
D50:	103.6 nm
D90:	179.2 nm

Stats: Mean +/- Standard Error

Mean:	119.5 +/- 1.1 nm
Mode:	86.3 +/- 3.1 nm
SD:	50.4 +/- 1.2 nm
D10:	73.8 +/- 1.0 nm
D50:	103.5 +/- 0.9 nm
D90:	179.5 +/- 1.7 nm
Concentration (Upgrade):	7.21e+10 +/- 2.68e+09 particles/ml
	130.7 +/- 4.4 particles/frame
	121.3 +/- 2.9 centres/frame

NANOSIGHT

2021.05.06 ECV 17PE 2021-05-06 15-17-30



Intensity / Size graph for Experiment:
2021.05.06 ECV 17PE 2021-05-06 15-17-30

Script Used: (Full Text):

SOP Standard Measurement 03-13-28PM 06May2021.txt

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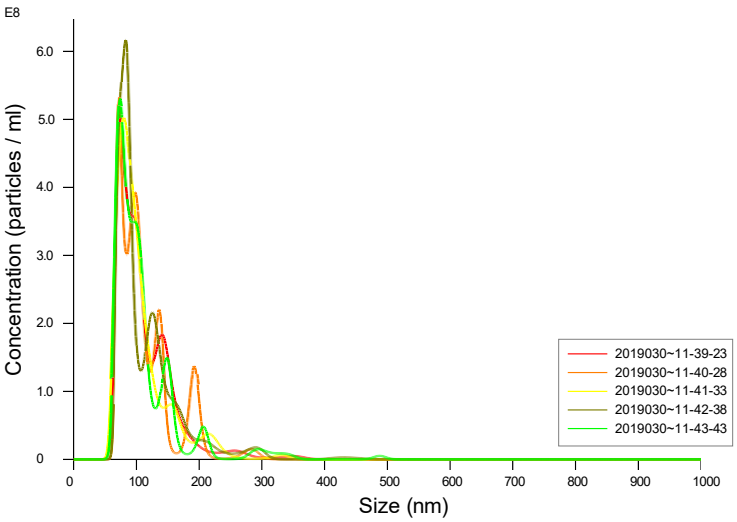
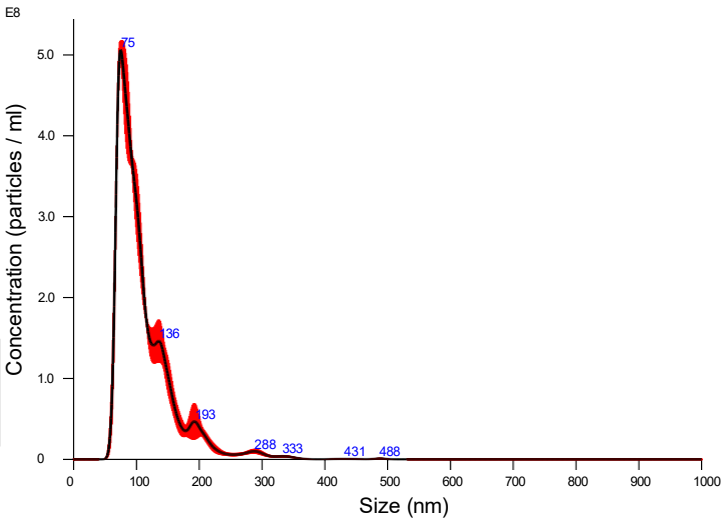
20190301_Exosomes_ECV20 1-100 2019-03-01 11-36-38



FTLA Concentration / Size graph for Experiment:
20190301_Exosomes_ECV20 1-100 2019-03-01 11-36-38



Averaged FTLA Concentration / Size for Experiment:
20190301_Exosomes_ECV20 1-100 2019-03-01 11-36-38
Error bars indicate + / -1 standard error of the mean

Included Files

20190301_Exosomes_ECV20 1-100 2019-03-01 11-39-23
20190301_Exosomes_ECV20 1-100 2019-03-01 11-40-28
20190301_Exosomes_ECV20 1-100 2019-03-01 11-41-33
20190301_Exosomes_ECV20 1-100 2019-03-01 11-42-38
20190301_Exosomes_ECV20 1-100 2019-03-01 11-43-43

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 11-08-36AM 01~
Time Captured:	11:36:38 01/03/2019
Operator:	
Pre-treatment:	1:100 dilution
Sample Name:	100 nm blue laser Flow cell
Diluent:	PBS
Remarks:	ECV 20 Exosomes

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	15
Slider Shutter:	1206
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	23.7 - 23.9 ºC
Viscosity:	(Water) 0.912 - 0.915 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 13.7 - 14.8 pix

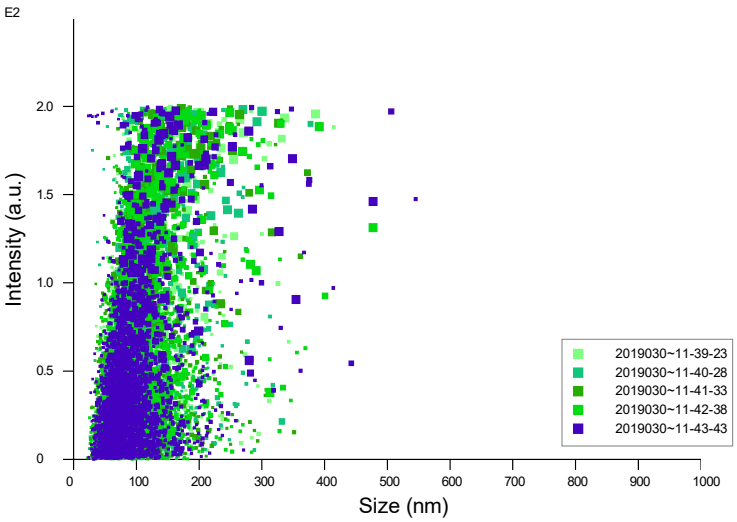
Results

Stats: Merged Data

Mean:	111.4 nm
Mode:	74.8 nm
SD:	48.6 nm
D10:	70.9 nm
D50:	96.4 nm
D90:	168.7 nm

Stats: Mean +/- Standard Error

Mean:	111.4 +/- 1.2 nm
Mode:	76.9 +/- 1.9 nm
SD:	48.3 +/- 2.4 nm
D10:	70.8 +/- 0.6 nm
D50:	96.3 +/- 1.3 nm
D90:	169.2 +/- 5.2 nm
Concentration (Upgrade):	2.79e+10 +/- 3.41e+08 particles/ml
	46.7 +/- 0.6 particles/frame
	46.1 +/- 0.6 centres/frame



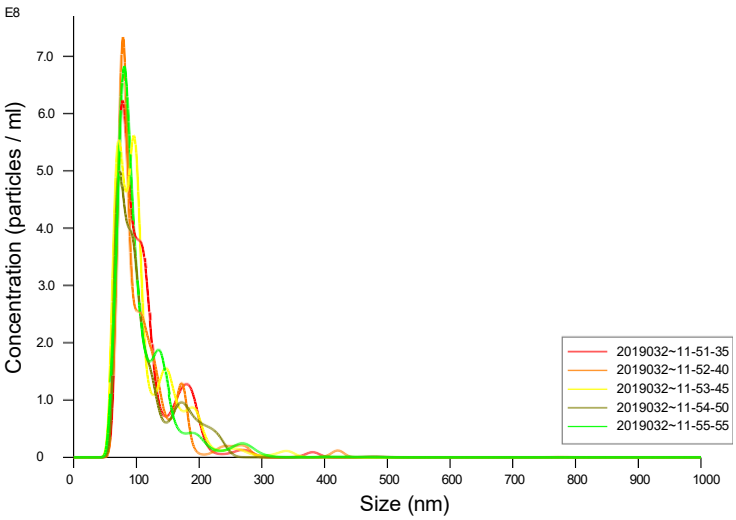
Intensity / Size graph for Experiment:
20190301_Exosomes_ECV20 1-100 2019-03-01 11-36-38

Script Used: (Full Text):

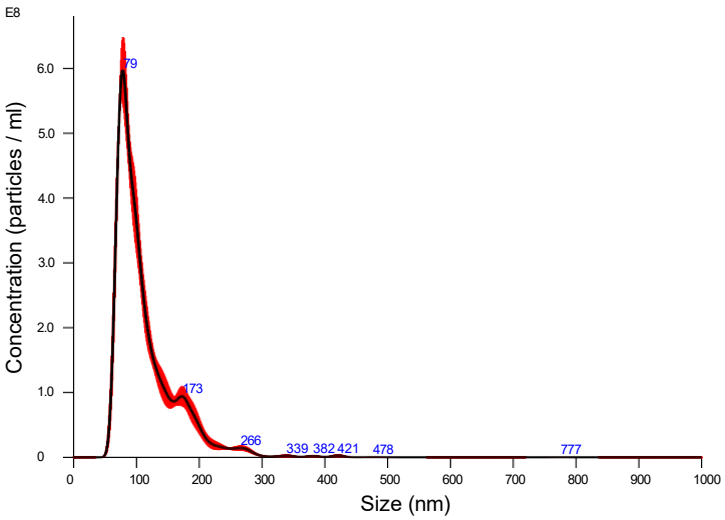
SOP Standard Measurement 11-08-36AM 01Mar2019.txt

NANOSIGHT

20190322_Exosomes_ECA217 1-50 2019-03-22 11-48-56



FTLA Concentration / Size graph for Experiment:
20190322_Exosomes_ECA217 1-50 2019-03-22 11-48-56



Averaged FTLA Concentration / Size for Experiment:
20190322_Exosomes_ECA217 1-50 2019-03-22 11-48-56
Error bars indicate + / -1 standard error of the mean

Included Files

20190322_Exosomes_ECA217 1-50 2019-03-22 11-51-35
20190322_Exosomes_ECA217 1-50 2019-03-22 11-52-40
20190322_Exosomes_ECA217 1-50 2019-03-22 11-53-45
20190322_Exosomes_ECA217 1-50 2019-03-22 11-54-50
20190322_Exosomes_ECA217 1-50 2019-03-22 11-55-55

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 11-48-40AM 22~
Time Captured:	11:48:56 22/03/2019
Operator:	Vi
Pre-treatment:	1:50
Sample Name:	Blue Laser
Diluent:	PBS
Remarks:	ECA 217 Exosomes

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	24.2 - 24.5 ºC
Viscosity:	(Water) 0.900 - 0.905 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 13.7 - 14.6 pix

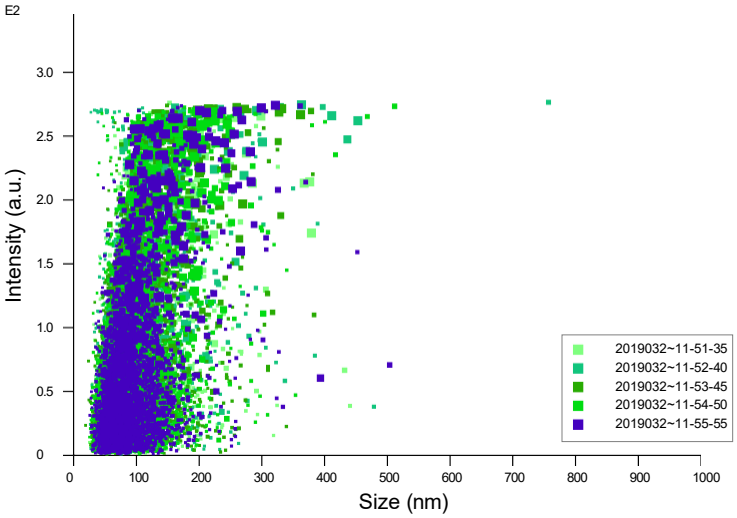
Results

Stats: Merged Data

Mean:	112.6 nm
Mode:	78.1 nm
SD:	50.3 nm
D10:	70.4 nm
D50:	96.3 nm
D90:	179.1 nm

Stats: Mean +/- Standard Error

Mean:	112.7 +/- 0.8 nm
Mode:	81.4 +/- 3.8 nm
SD:	50.3 +/- 1.4 nm
D10:	70.4 +/- 0.9 nm
D50:	96.2 +/- 1.2 nm
D90:	178.2 +/- 3.7 nm
Concentration (Upgrade):	3.31e+10 +/- 1.36e+09 particles/ml
	54.2 +/- 2.1 particles/frame
	53.9 +/- 1.8 centres/frame



Intensity / Size graph for Experiment:
20190322_Exosomes_ECA217 1-50 2019-03-22 11-48-56

Script Used: (Full Text):

SOP Standard Measurement 11-48-40AM 22Mar2019.txt

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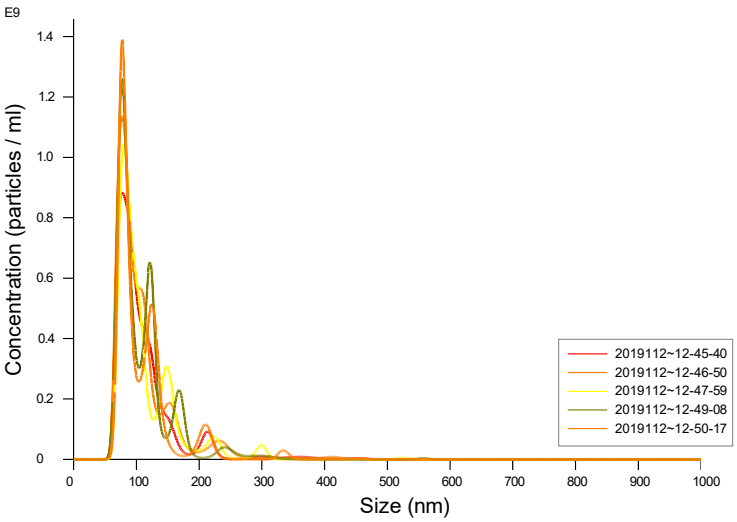
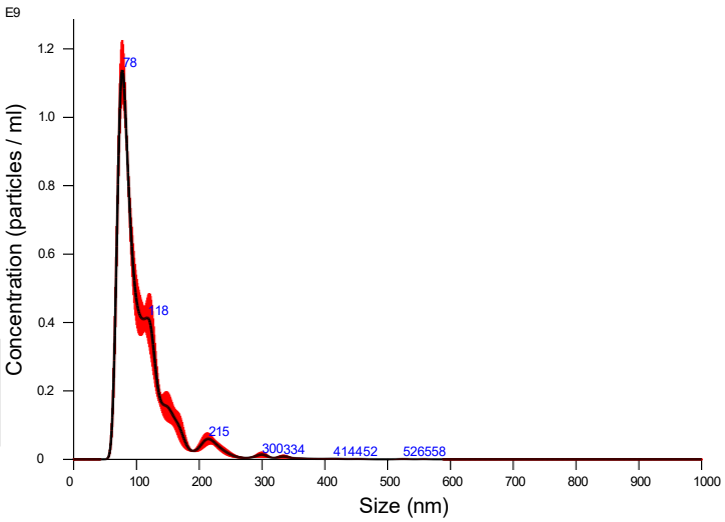
20191122_Exosomes_ECA208 2019-11-22 12-42-22



FTLA Concentration / Size graph for Experiment:
20191122_Exosomes_ECA208 2019-11-22 12-42-22



Averaged FTLA Concentration / Size for Experiment:
20191122_Exosomes_ECA208 2019-11-22 12-42-22
Error bars indicate + / -1 standard error of the mean

Included Files

20191122_Exosomes_ECA208 2019-11-22 12-45-40
20191122_Exosomes_ECA208 2019-11-22 12-46-50
20191122_Exosomes_ECA208 2019-11-22 12-47-59
20191122_Exosomes_ECA208 2019-11-22 12-49-08
20191122_Exosomes_ECA208 2019-11-22 12-50-17

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 12-38-37PM 22~
Time Captured:	12:42:22 22/11/2019
Operator:	Babsi
Pre-treatment:	1:50
Sample Name:	Exosomes, ECA208
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue405
Camera Level:	15
Slider Shutter:	1206
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	21.5 - 21.6 ºC
Viscosity:	(Water) 0.962 - 0.965 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 13.4 - 14.1 pix

Results

Stats: Merged Data

Mean:	109.5 nm
Mode:	77.4 nm
SD:	48.9 nm
D10:	71.7 nm
D50:	93.8 nm
D90:	162.0 nm

Stats: Mean +/- Standard Error

Mean:	109.5 +/- 1.0 nm
Mode:	77.5 +/- 0.5 nm
SD:	48.8 +/- 0.8 nm
D10:	71.7 +/- 0.6 nm
D50:	93.5 +/- 1.0 nm
D90:	159.4 +/- 3.4 nm
Concentration (Upgrade):	5.11e+10 +/- 1.39e+09 particles/ml
	109.4 +/- 2.5 particles/frame
	104.3 +/- 1.9 centres/frame

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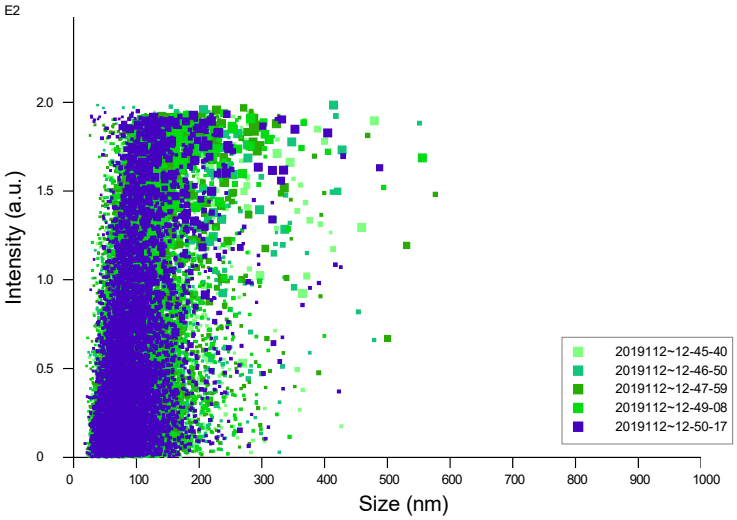
20191122_Exosomes_ECA208 2019-11-22 12-42-22



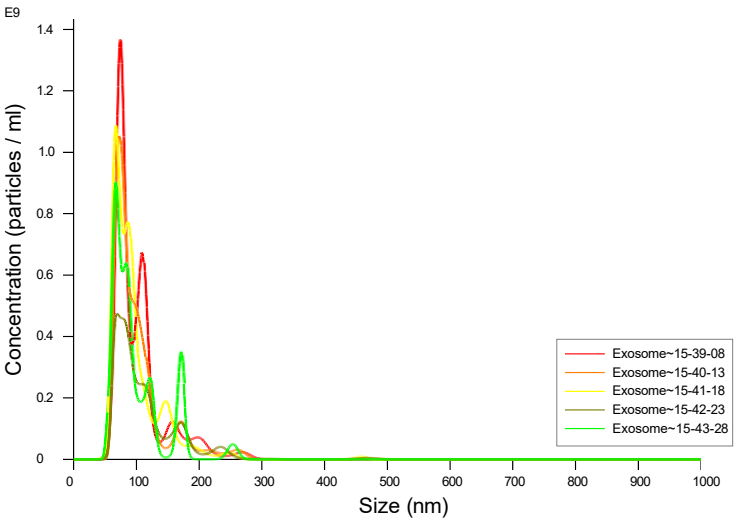
Intensity / Size graph for Experiment:
20191122_Exosomes_ECA208 2019-11-22 12-42-22

Script Used: (Full Text):

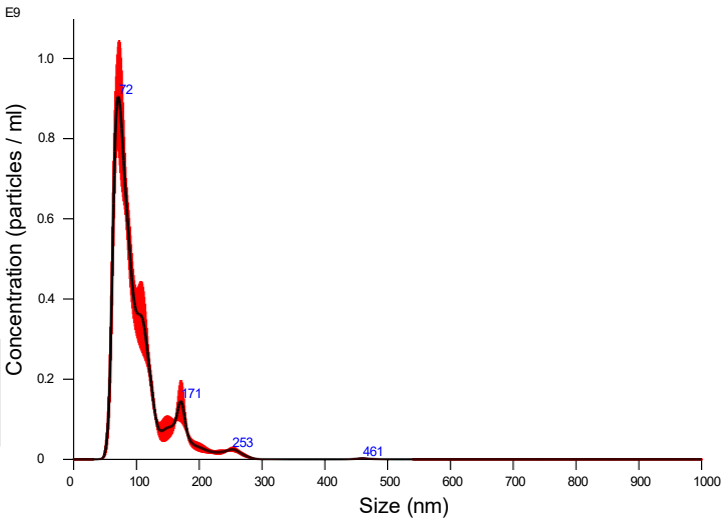
SOP Standard Measurement 12-38-37PM 22Nov2019.txt

NANOSIGHT

Exosomen_ECV 56_1 100 2019-02-15 15-36-23



FTLA Concentration / Size graph for Experiment:
Exosomen_ECV 56_1 100 2019-02-15 15-36-23



Averaged FTLA Concentration / Size for Experiment:
Exosomen_ECV 56_1 100 2019-02-15 15-36-23
Error bars indicate + / -1 standard error of the mean

Included Files

Exosomen_ECV 56_1 100 2019-02-15 15-39-08
Exosomen_ECV 56_1 100 2019-02-15 15-40-13
Exosomen_ECV 56_1 100 2019-02-15 15-41-18
Exosomen_ECV 56_1 100 2019-02-15 15-42-23
Exosomen_ECV 56_1 100 2019-02-15 15-43-28

Details

NTA Version:	NTA 3.2 Dev Build 3.2.16
Script Used:	SOP Standard Measurement 03-34-46PM 15~
Time Captured:	15:36:23 15/02/2019
Operator:	Vi
Pre-treatment:	1:100
Sample Name:	Blue Laser
Diluent:	PBS
Remarks:	ECV 56

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	16
Slider Shutter:	1300
Slider Gain:	512
FPS	25.0
Number of Frames:	1498
Temperature:	22.6 - 22.7 ºC
Viscosity:	(Water) 0.937 - 0.940 cP
Dilution factor:	1 x 10e2
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 13.4 - 15.1 pix

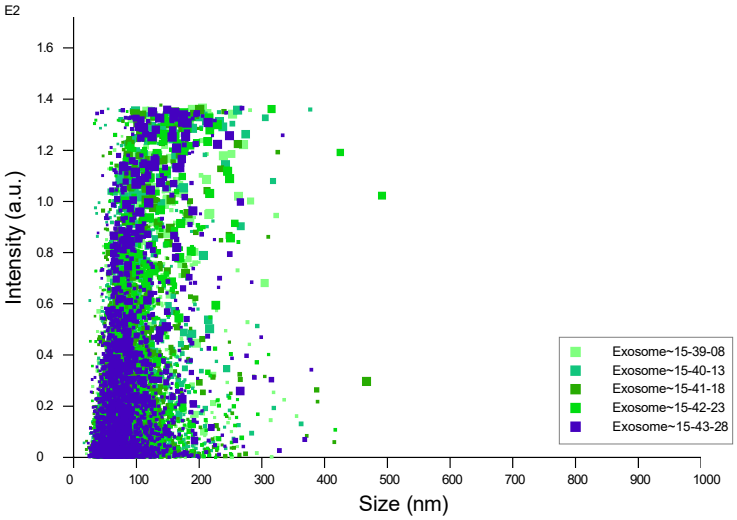
Results

Stats: Merged Data

Mean:	101.6 nm
Mode:	71.0 nm
SD:	44.4 nm
D10:	64.2 nm
D50:	86.0 nm
D90:	164.8 nm

Stats: Mean +/- Standard Error

Mean:	102.3 +/- 2.4 nm
Mode:	70.0 +/- 1.5 nm
SD:	44.6 +/- 2.1 nm
D10:	64.4 +/- 1.1 nm
D50:	86.9 +/- 1.9 nm
D90:	163.7 +/- 4.4 nm
Concentration (Upgrade):	4.27e+010 +/- 3.67e+009 particles/ml
	35.6 +/- 2.7 particles/frame
	36.4 +/- 2.5 centres/frame



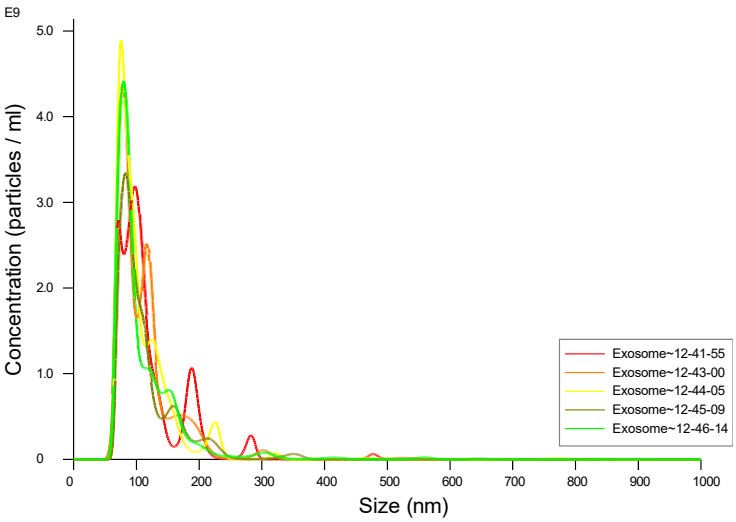
Intensity / Size graph for Experiment:
Exosomen_ECV 56_1 100 2019-02-15 15-36-23

Script Used: (Full Text):

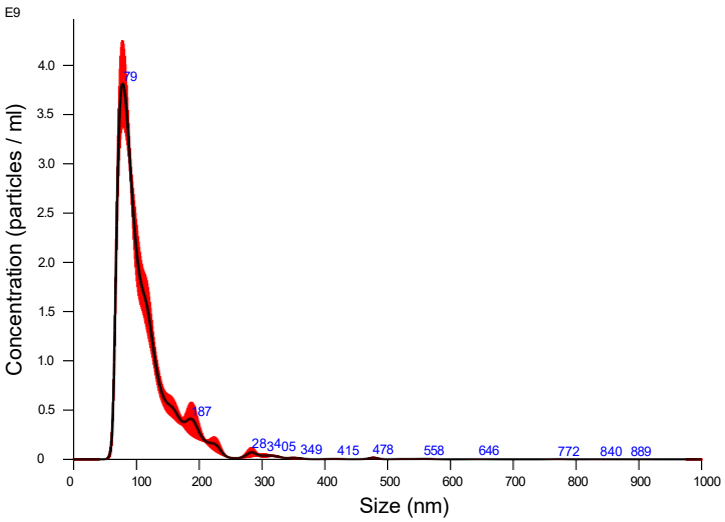
SOP Standard Measurement 03-34-46PM 15Feb2019.txt

NANOSIGHT

Exosomes_ECV 84_1 200 2019-02-22 12-38-59



FTLA Concentration / Size graph for Experiment:
Exosomes_ECV 84_1 200 2019-02-22 12-38-59



Averaged FTLA Concentration / Size for Experiment:
Exosomes_ECV 84_1 200 2019-02-22 12-38-59
Error bars indicate + / -1 standard error of the mean

Included Files

Exosomes_ECV 84_1 200 2019-02-22 12-41-55
Exosomes_ECV 84_1 200 2019-02-22 12-43-00
Exosomes_ECV 84_1 200 2019-02-22 12-44-05
Exosomes_ECV 84_1 200 2019-02-22 12-45-09
Exosomes_ECV 84_1 200 2019-02-22 12-46-14

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 12-38-16PM 22~
Time Captured:	12:38:59 22/02/2019
Operator:	Vi
Pre-treatment:	1:200
Sample Name:	Blue Laser
Diluent:	PBS
Remarks:	ECV 84

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	22.9 - 23.0 ºC
Viscosity:	(Water) 0.930 - 0.932 cP
Dilution factor:	2 x 10e2
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 13.1 - 13.9 pix

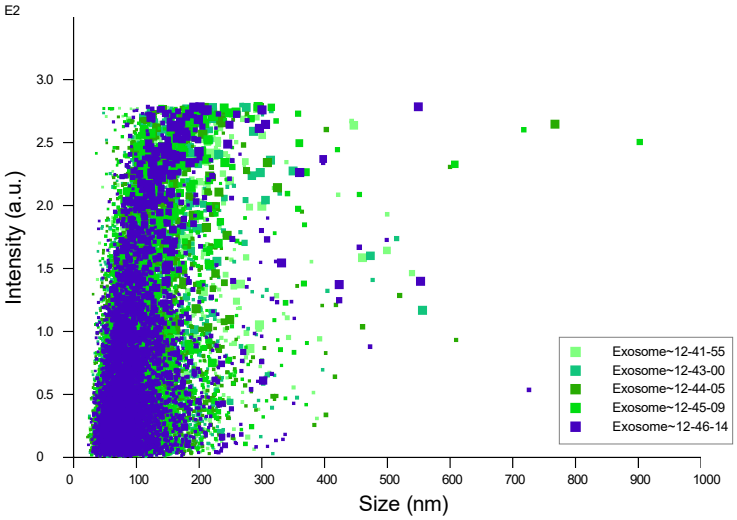
Results

Stats: Merged Data

Mean:	113.1 nm
Mode:	78.0 nm
SD:	54.7 nm
D10:	72.3 nm
D50:	96.7 nm
D90:	175.4 nm

Stats: Mean +/- Standard Error

Mean:	113.3 +/- 1.5 nm
Mode:	82.8 +/- 3.9 nm
SD:	54.7 +/- 1.0 nm
D10:	72.4 +/- 0.5 nm
D50:	96.6 +/- 1.8 nm
D90:	172.4 +/- 5.3 nm
Concentration (Upgrade):	2.02e+11 +/- 8.53e+09 particles/ml
	78.2 +/- 3.1 particles/frame
	75.0 +/- 2.5 centres/frame



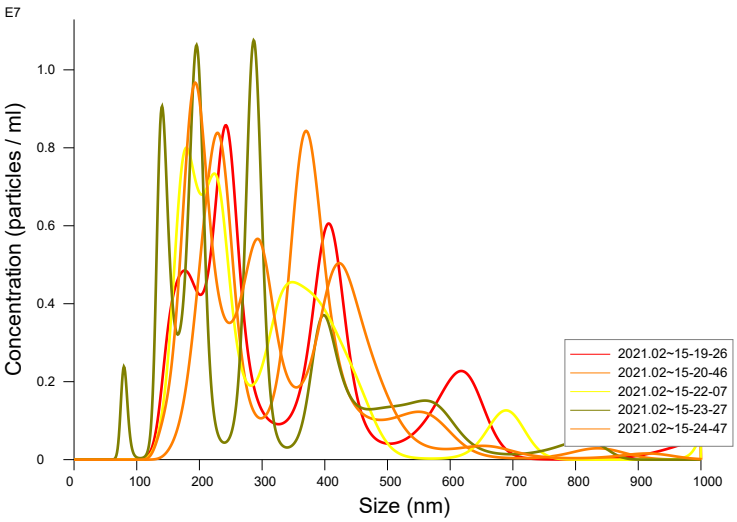
Intensity / Size graph for Experiment:
Exosomes_ECV 84_1 200 2019-02-22 12-38-59

Script Used: (Full Text):

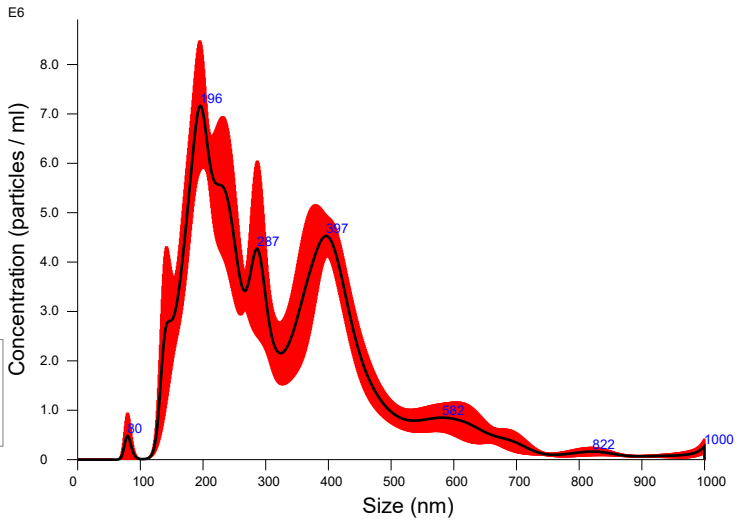
SOP Standard Measurement 12-38-16PM 22Feb2019.txt

NANOSIGHT

2021.02.10 MV ECA212 2021-02-10 15-16-45



FTLA Concentration / Size graph for Experiment:
2021.02.10 MV ECA212 2021-02-10 15-16-45



Averaged FTLA Concentration / Size for Experiment:
2021.02.10 MV ECA212 2021-02-10 15-16-45
Error bars indicate + / -1 standard error of the mean

Included Files

2021.02.10 MV ECA212 2021-02-10 15-19-26
2021.02.10 MV ECA212 2021-02-10 15-20-46
2021.02.10 MV ECA212 2021-02-10 15-22-07
2021.02.10 MV ECA212 2021-02-10 15-23-27
2021.02.10 MV ECA212 2021-02-10 15-24-47

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 03-14-55PM 10~
Time Captured:	15:16:45 10/02/2021
Operator:	Hanna
Pre-treatment:	1:10
Sample Name:	MV ECa212
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	8 - 9
Slider Shutter:	317 - 607
Slider Gain:	15
FPS	25.0
Number of Frames:	1498
Temperature:	25.3 - 25.5 ºC
Viscosity:	(Water) 0.879 - 0.883 cP
Dilution factor:	1 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 8.8 - 12.0 pix

Results

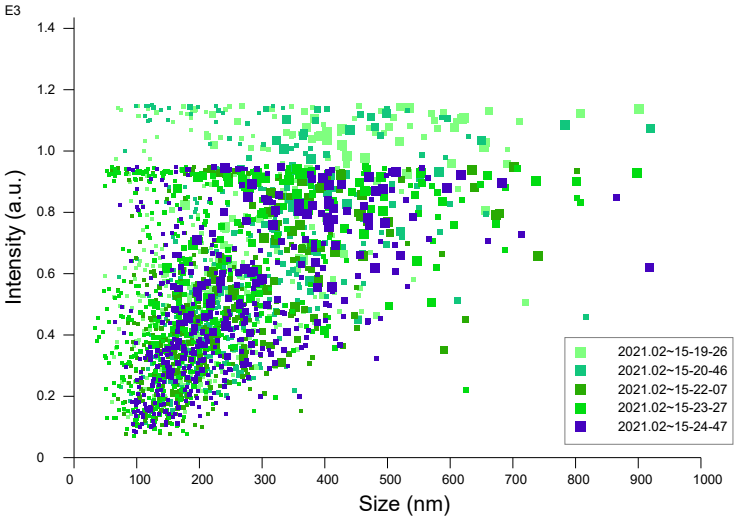
Stats: Merged Data

Mean:	329.6 nm
Mode:	195.7 nm
SD:	151.6 nm
D10:	175.2 nm
D50:	293.5 nm
D90:	531.0 nm

Stats: Mean +/- Standard Error

Mean:	329.9 +/- 8.0 nm
Mode:	254.3 +/- 34.6 nm
SD:	149.9 +/- 8.3 nm
D10:	176.1 +/- 10.4 nm
D50:	299.4 +/- 13.0 nm
D90:	524.7 +/- 26.9 nm
Concentration (Upgrade):	1.53e+09 +/- 3.33e+07 particles/ml
	14.4 +/- 0.6 particles/frame
	18.8 +/- 1.0 centres/frame

Concentration measurements may require some caution due to noise
See summary file for more info



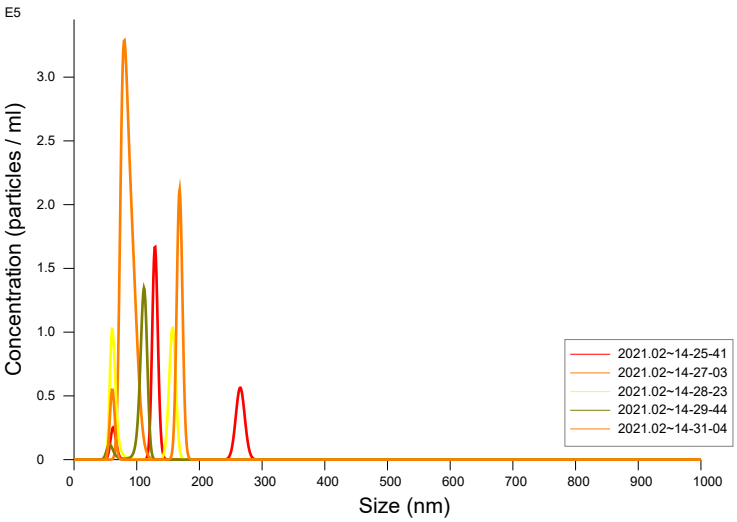
Intensity / Size graph for Experiment:
2021.02.10 MV ECA212 2021-02-10 15-16-45

Script Used: (Full Text):

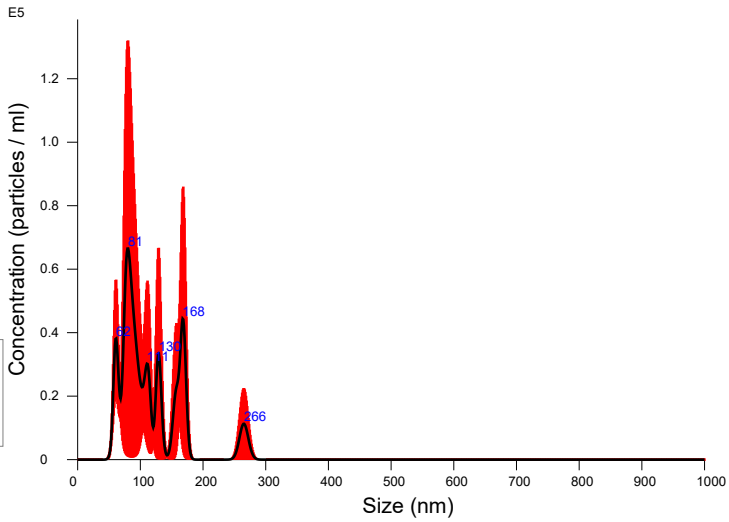
SOP Standard Measurement 03-14-55PM 10Feb2021.txt

NANOSIGHT

2021.02.10 PBS 2021-02-10 14-22-51



FTLA Concentration / Size graph for Experiment:
2021.02.10 PBS 2021-02-10 14-22-51



Averaged FTLA Concentration / Size for Experiment:
2021.02.10 PBS 2021-02-10 14-22-51
Error bars indicate + / -1 standard error of the mean

Included Files

2021.02.10 PBS 2021-02-10 14-25-41
2021.02.10 PBS 2021-02-10 14-27-03
2021.02.10 PBS 2021-02-10 14-28-23
2021.02.10 PBS 2021-02-10 14-29-44
2021.02.10 PBS 2021-02-10 14-31-04

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 02-22-19PM 10~
Time Captured:	14:22:51 10/02/2021
Operator:	Hanna
Pre-treatment:	
Sample Name:	PBS
Diluent:	
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	24.7 - 25.0 ºC
Viscosity:	(Water) 0.888 - 0.894 cP
Dilution factor:	Dilution not recorded
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 14.2 pix

Results

Stats: Merged Data

Mean:	116.1 nm
Mode:	80.0 nm
SD:	50.3 nm
D10:	66.3 nm
D50:	99.9 nm
D90:	170.4 nm

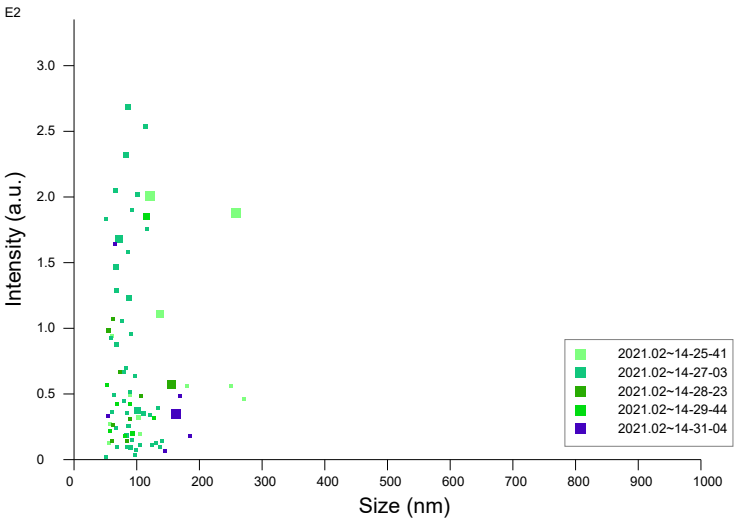
Stats: Mean +/- Standard Error

Mean:	124.1 +/- 14.8 nm
Mode:	129.2 +/- 15.9 nm
SD:	36.8 +/- 11.0 nm
D10:	82.1 +/- 11.6 nm
D50:	128.3 +/- 14.5 nm
D90:	164.1 +/- 29.5 nm
Concentration (Upgrade):	3.64e+06 +/- 9.79e+05 particles/ml
	0.3 +/- 0.1 particles/frame
	0.3 +/- 0.1 centres/frame

Concentration measurements may be unreliable
See summary file for more info

NANOSIGHT

2021.02.10 PBS 2021-02-10 14-22-51



Intensity / Size graph for Experiment:
2021.02.10 PBS 2021-02-10 14-22-51

Script Used: (Full Text):

SOP Standard Measurement 02-22-19PM 10Feb2021.txt

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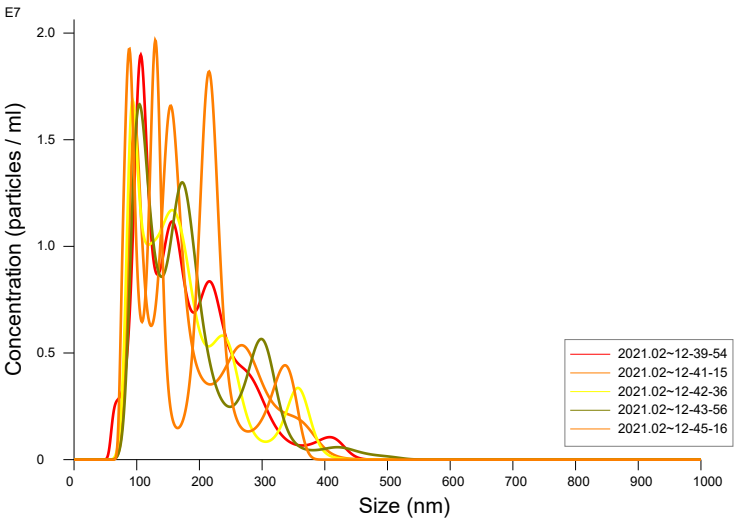
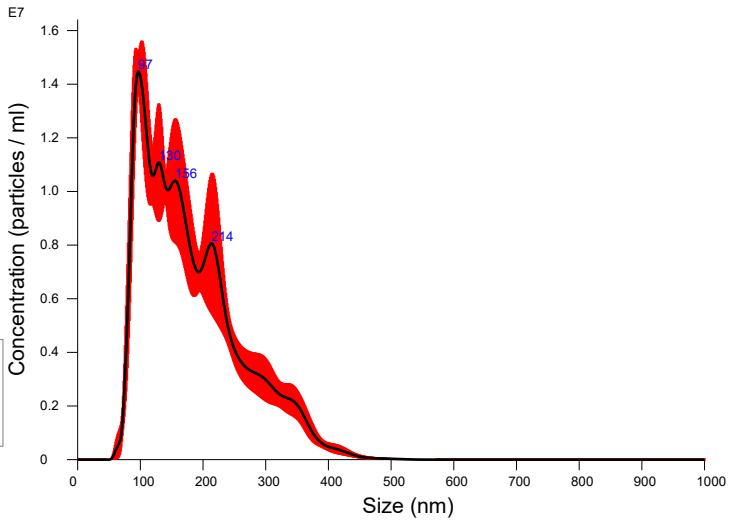
2021.02.25 MV ECA42 2021-02-25 12-37-11



FTLA Concentration / Size graph for Experiment:
2021.02.25 MV ECA42 2021-02-25 12-37-11



Averaged FTLA Concentration / Size for Experiment:
2021.02.25 MV ECA42 2021-02-25 12-37-11
Error bars indicate + / -1 standard error of the mean

Included Files

2021.02.25 MV ECA42 2021-02-25 12-39-54
2021.02.25 MV ECA42 2021-02-25 12-41-15
2021.02.25 MV ECA42 2021-02-25 12-42-36
2021.02.25 MV ECA42 2021-02-25 12-43-56
2021.02.25 MV ECA42 2021-02-25 12-45-16

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 12-36-32PM 25~
Time Captured:	12:37:11 25/02/2021
Operator:	Hanna
Pre-treatment:	1:10
Sample Name:	MV ECA 42
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	13
Slider Shutter:	1232
Slider Gain:	219
FPS	25.0
Number of Frames:	1498
Temperature:	25.4 - 25.6 ºC
Viscosity:	(Water) 0.876 - 0.881 cP
Dilution factor:	1 x 10e1
Syringe Pump Speed:	100

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 11.8 - 13.8 pix

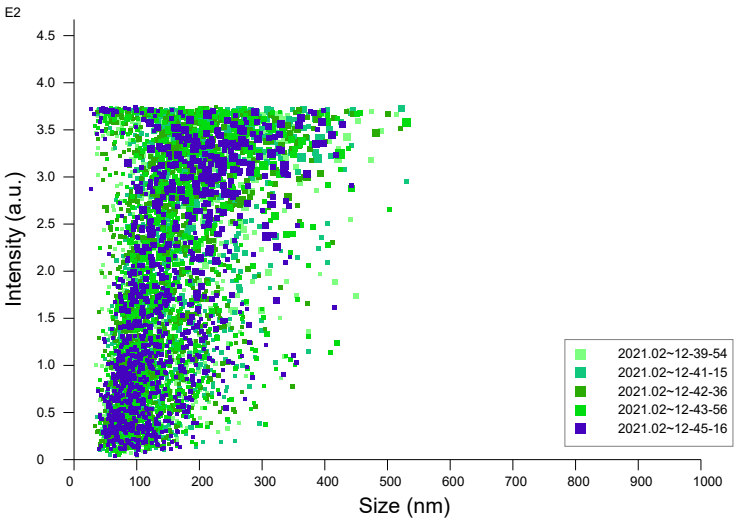
Results

Stats: Merged Data

Mean:	181.6 nm
Mode:	97.0 nm
SD:	78.9 nm
D10:	94.7 nm
D50:	164.2 nm
D90:	300.4 nm

Stats: Mean +/- Standard Error

Mean:	181.7 +/- 1.8 nm
Mode:	117.6 +/- 10.9 nm
SD:	78.8 +/- 0.8 nm
D10:	95.1 +/- 2.2 nm
D50:	167.3 +/- 4.0 nm
D90:	299.1 +/- 4.3 nm
Concentration (Upgrade):	1.96e+09 +/- 2.91e+07 particles/ml
	20.7 +/- 0.3 particles/frame
	22.5 +/- 0.5 centres/frame



Intensity / Size graph for Experiment:
2021.02.25 MV ECA42 2021-02-25 12-37-11

Script Used: (Full Text):

SOP Standard Measurement 12-36-32PM 25Feb2021.txt

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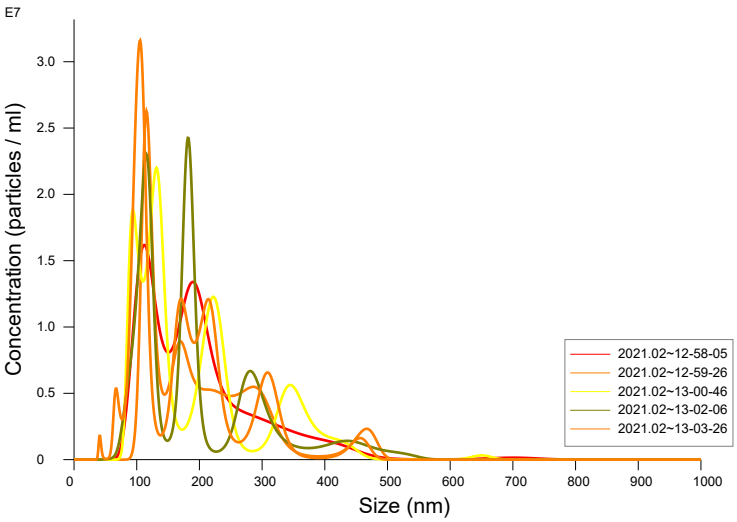
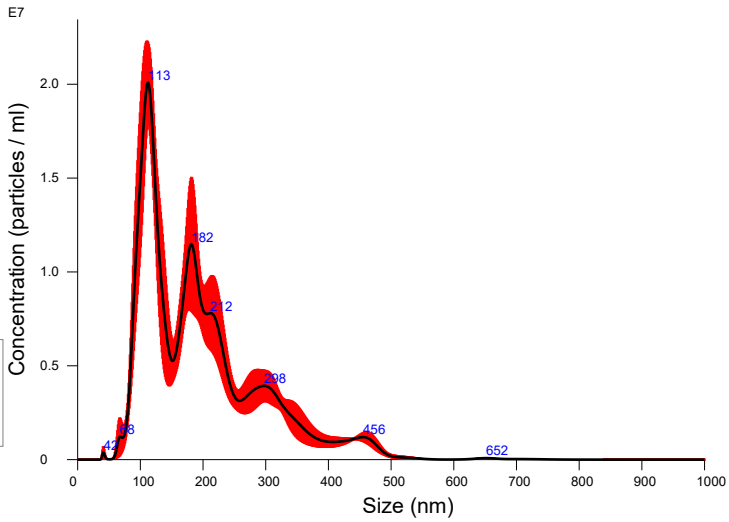
2021.02.25 MV ECA158 2021-02-25 12-55-25



FTLA Concentration / Size graph for Experiment:
2021.02.25 MV ECA158 2021-02-25 12-55-25



Averaged FTLA Concentration / Size for Experiment:
2021.02.25 MV ECA158 2021-02-25 12-55-25
Error bars indicate + / -1 standard error of the mean

Included Files

2021.02.25 MV ECA158 2021-02-25 12-58-05
2021.02.25 MV ECA158 2021-02-25 12-59-26
2021.02.25 MV ECA158 2021-02-25 13-00-46
2021.02.25 MV ECA158 2021-02-25 13-02-06
2021.02.25 MV ECA158 2021-02-25 13-03-26

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 12-55-23PM 25~
Time Captured:	12:55:25 25/02/2021
Operator:	Hanna
Pre-treatment:	1:10
Sample Name:	MV ECA 158
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	12
Slider Shutter:	1200
Slider Gain:	146
FPS	25.0
Number of Frames:	1498
Temperature:	25.6 - 25.9 ºC
Viscosity:	(Water) 0.871 - 0.876 cP
Dilution factor:	1 x 10e1
Syringe Pump Speed:	100

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 11.3 - 14.7 pix

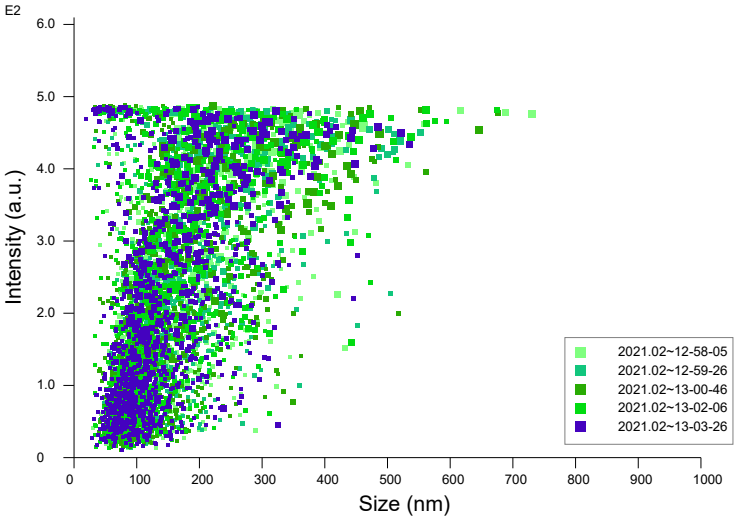
Results

Stats: Merged Data

Mean:	196.0 nm
Mode:	112.2 nm
SD:	97.3 nm
D10:	100.6 nm
D50:	176.0 nm
D90:	331.8 nm

Stats: Mean +/- Standard Error

Mean:	196.4 +/- 4.2 nm
Mode:	129.3 +/- 13.9 nm
SD:	96.8 +/- 2.6 nm
D10:	101.7 +/- 3.2 nm
D50:	172.7 +/- 5.1 nm
D90:	330.9 +/- 8.0 nm
Concentration (Upgrade):	2.10e+09 +/- 8.90e+07 particles/ml
	20.3 +/- 0.6 particles/frame
	22.2 +/- 0.5 centres/frame



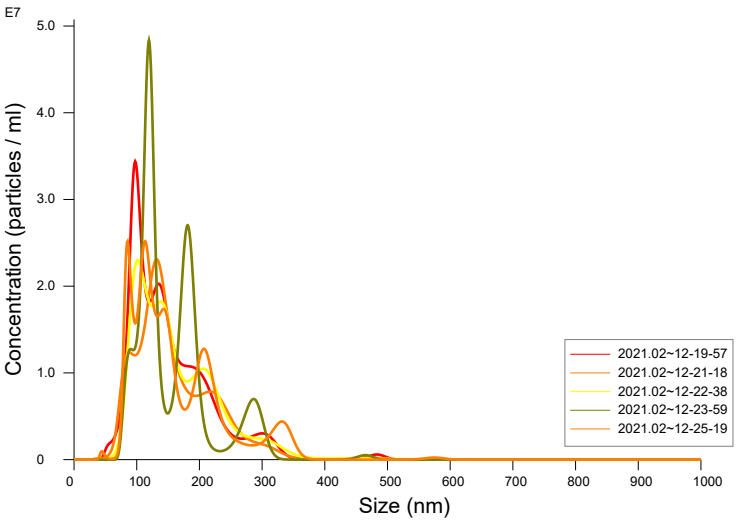
Intensity / Size graph for Experiment:
2021.02.25 MV ECA158 2021-02-25 12-55-25

Script Used: (Full Text):

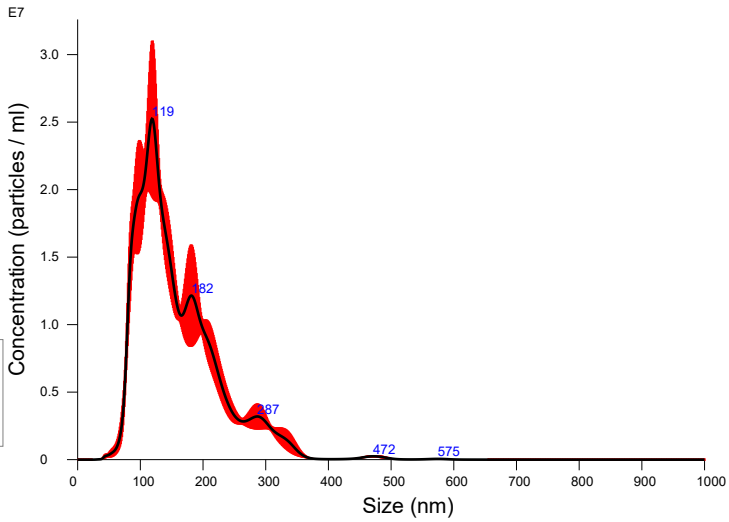
SOP Standard Measurement 12-55-23PM 25Feb2021.txt

NANOSIGHT

2021.02.25 MV ECV158 2021-02-25 12-17-13



FTLA Concentration / Size graph for Experiment:
2021.02.25 MV ECV158 2021-02-25 12-17-13



Averaged FTLA Concentration / Size for Experiment:
2021.02.25 MV ECV158 2021-02-25 12-17-13
Error bars indicate + / -1 standard error of the mean

Included Files

2021.02.25 MV ECV158 2021-02-25 12-19-57
2021.02.25 MV ECV158 2021-02-25 12-21-18
2021.02.25 MV ECV158 2021-02-25 12-22-38
2021.02.25 MV ECV158 2021-02-25 12-23-59
2021.02.25 MV ECV158 2021-02-25 12-25-19

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 12-16-38PM 25~
Time Captured:	12:17:13 25/02/2021
Operator:	Hanna
Pre-treatment:	1:10
Sample Name:	MV ECv158
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	25.0 - 25.2 ºC
Viscosity:	(Water) 0.884 - 0.889 cP
Dilution factor:	1 x 10e1
Syringe Pump Speed:	100

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 12.3 - 13.9 pix

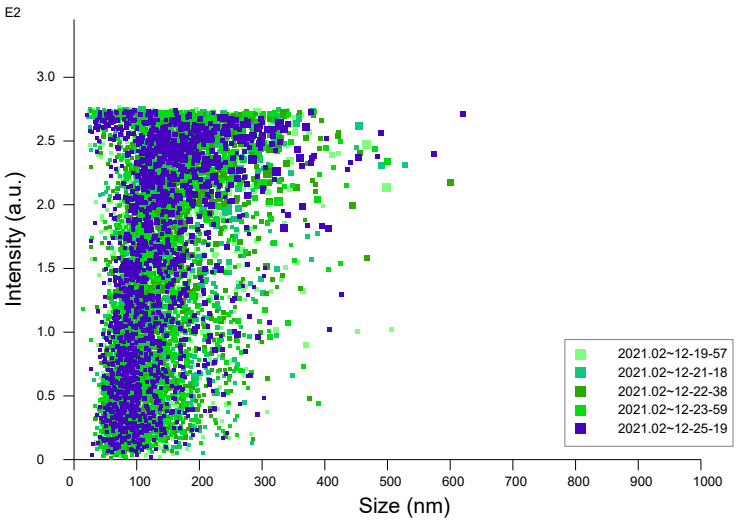
Results

Stats: Merged Data

Mean:	159.1 nm
Mode:	118.8 nm
SD:	66.8 nm
D10:	91.6 nm
D50:	140.7 nm
D90:	250.7 nm

Stats: Mean +/- Standard Error

Mean:	159.2 +/- 1.8 nm
Mode:	112.7 +/- 6.2 nm
SD:	66.6 +/- 2.1 nm
D10:	92.2 +/- 2.1 nm
D50:	140.2 +/- 2.1 nm
D90:	253.2 +/- 5.5 nm
Concentration (Upgrade):	2.58e+09 +/- 7.70e+07 particles/ml
	28.2 +/- 0.8 particles/frame
	31.2 +/- 0.9 centres/frame



Intensity / Size graph for Experiment:
2021.02.25 MV ECV158 2021-02-25 12-17-13

Script Used: (Full Text):

SOP Standard Measurement 12-16-38PM 25Feb2021.txt

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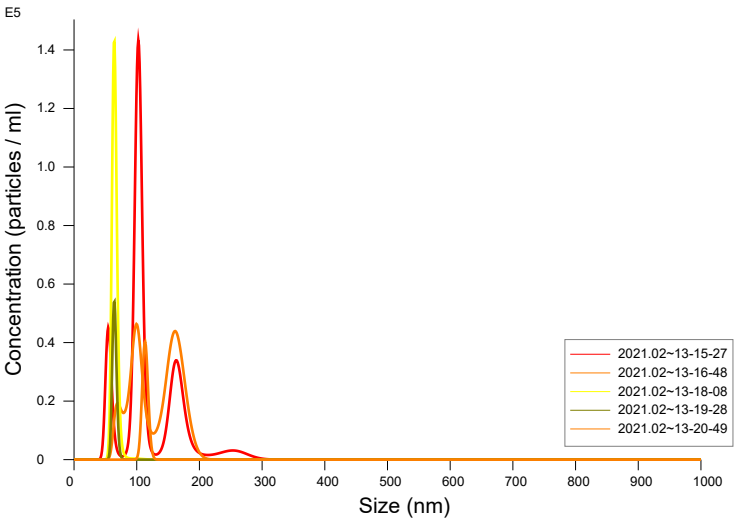
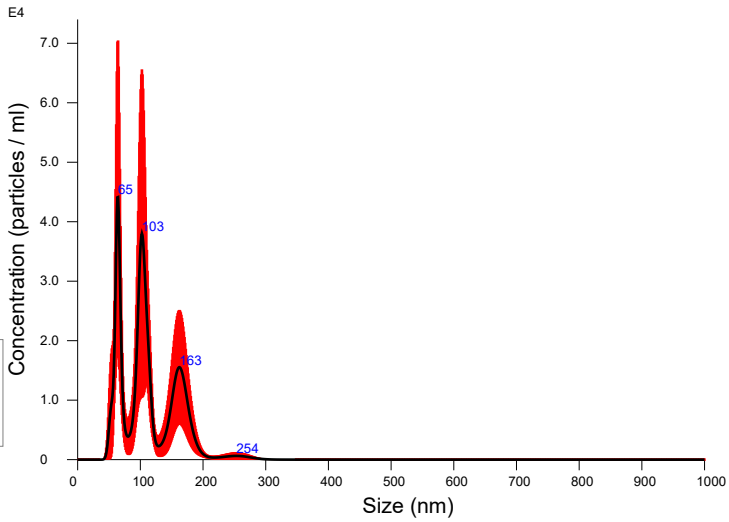
2021.02.25 PBS 2021-02-25 13-12-44



FTLA Concentration / Size graph for Experiment:
2021.02.25 PBS 2021-02-25 13-12-44



Averaged FTLA Concentration / Size for Experiment:
2021.02.25 PBS 2021-02-25 13-12-44
Error bars indicate + / -1 standard error of the mean

Included Files

2021.02.25 PBS 2021-02-25 13-15-27
2021.02.25 PBS 2021-02-25 13-16-48
2021.02.25 PBS 2021-02-25 13-18-08
2021.02.25 PBS 2021-02-25 13-19-28
2021.02.25 PBS 2021-02-25 13-20-49

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 01-12-27PM 25~
Time Captured:	13:12:44 25/02/2021
Operator:	Hanna
Pre-treatment:	
Sample Name:	PBS
Diluent:	
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	12
Slider Shutter:	1200
Slider Gain:	146
FPS	25.0
Number of Frames:	1498
Temperature:	25.9 - 26.1 ºC
Viscosity:	(Water) 0.866 - 0.870 cP
Dilution factor:	Dilution not recorded
Syringe Pump Speed:	100

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 14.2 pix

Results

Stats: Merged Data

Mean:	111.7 nm
Mode:	64.0 nm
SD:	44.1 nm
D10:	61.8 nm
D50:	103.7 nm
D90:	170.9 nm

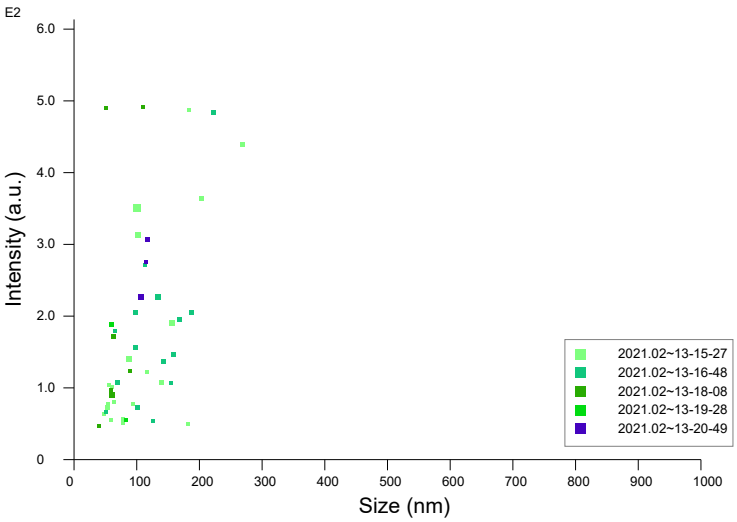
Stats: Mean +/- Standard Error

Mean:	98.4 +/- 14.0 nm
Mode:	88.7 +/- 10.3 nm
SD:	19.5 +/- 9.3 nm
D10:	73.1 +/- 9.5 nm
D50:	96.6 +/- 14.0 nm
D90:	121.9 +/- 23.6 nm
Concentration (Upgrade):	1.92e+06 +/- 7.24e+05 particles/ml
	0.2 +/- 0.1 particles/frame
	0.2 +/- 0.1 centres/frame

Concentration measurements may be unreliable
See summary file for more info

NANOSIGHT

2021.02.25 PBS 2021-02-25 13-12-44



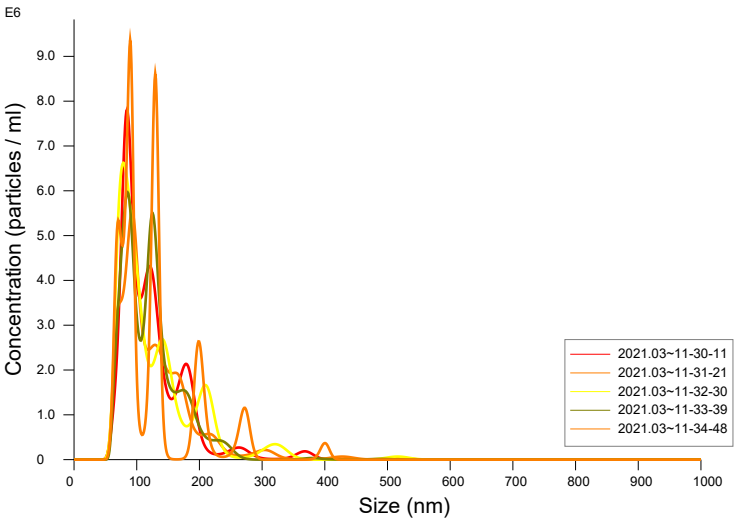
Intensity / Size graph for Experiment:
2021.02.25 PBS 2021-02-25 13-12-44

Script Used: (Full Text):

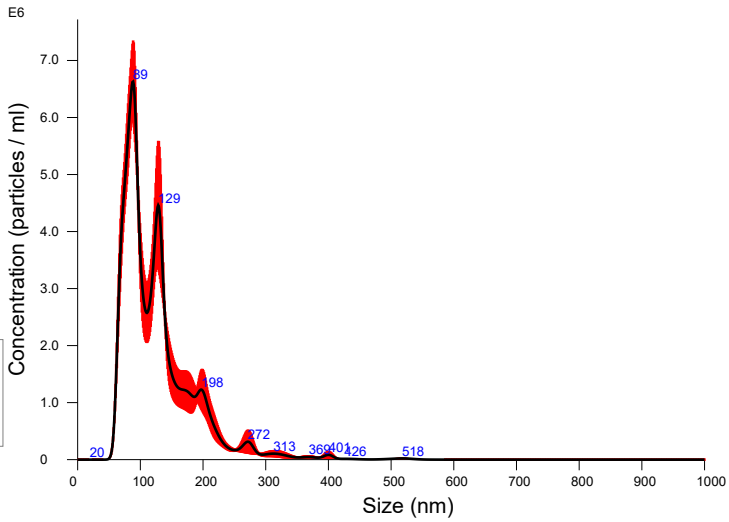
SOP Standard Measurement 01-12-27PM 25Feb2021.txt

NANOSIGHT

2021.03.12 ECA 10PE (2) 2021-03-12 11-27-05



FTLA Concentration / Size graph for Experiment:
2021.03.12 ECA 10PE (2) 2021-03-12 11-27-05



Averaged FTLA Concentration / Size for Experiment:
2021.03.12 ECA 10PE (2) 2021-03-12 11-27-05
Error bars indicate + / -1 standard error of the mean

Included Files

2021.03.12 ECA 10PE (2) 2021-03-12 11-30-11
2021.03.12 ECA 10PE (2) 2021-03-12 11-31-21
2021.03.12 ECA 10PE (2) 2021-03-12 11-32-30
2021.03.12 ECA 10PE (2) 2021-03-12 11-33-39
2021.03.12 ECA 10PE (2) 2021-03-12 11-34-48

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 11-25-53AM 12~
Time Captured:	11:27:05 12/03/2021
Operator:	Hanna
Pre-treatment:	1:50
Sample Name:	ECA 10PE
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	15
Slider Shutter:	1206
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	24.5 - 24.7 ºC
Viscosity:	(Water) 0.895 - 0.899 cP
Dilution factor:	Dilution not recorded
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 13.3 - 15.0 pix

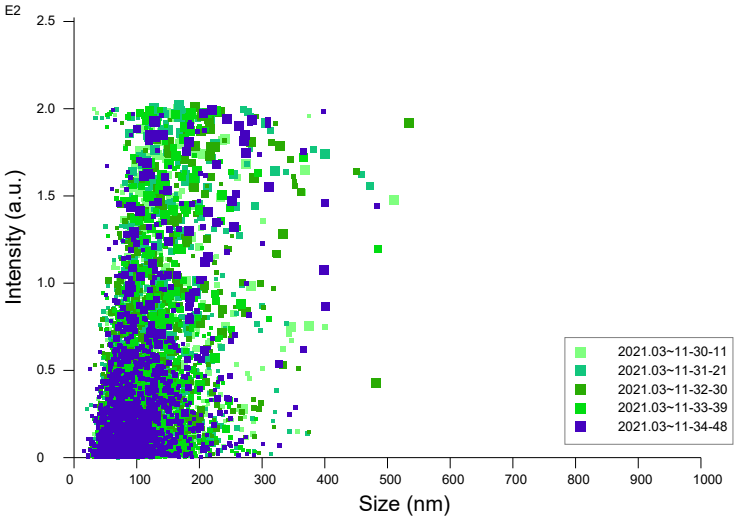
Results

Stats: Merged Data

Mean:	126.2 nm
Mode:	88.1 nm
SD:	59.1 nm
D10:	73.5 nm
D50:	111.1 nm
D90:	199.4 nm

Stats: Mean +/- Standard Error

Mean:	126.2 +/- 1.1 nm
Mode:	86.1 +/- 2.4 nm
SD:	58.5 +/- 3.5 nm
D10:	73.6 +/- 1.0 nm
D50:	110.2 +/- 2.0 nm
D90:	196.6 +/- 5.7 nm
Concentration (Upgrade):	4.47e+08 +/- 1.22e+07 particles/ml
	31.9 +/- 0.6 particles/frame
	33.5 +/- 0.7 centres/frame



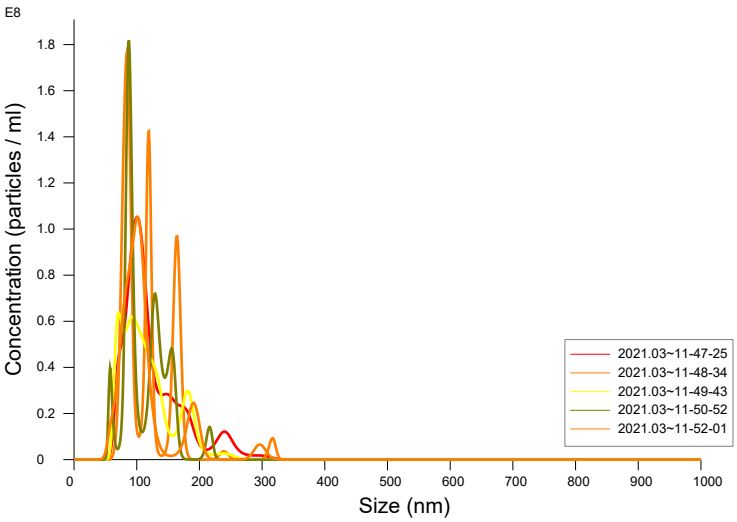
Intensity / Size graph for Experiment:
2021.03.12 ECA 10PE (2) 2021-03-12 11-27-05

Script Used: (Full Text):

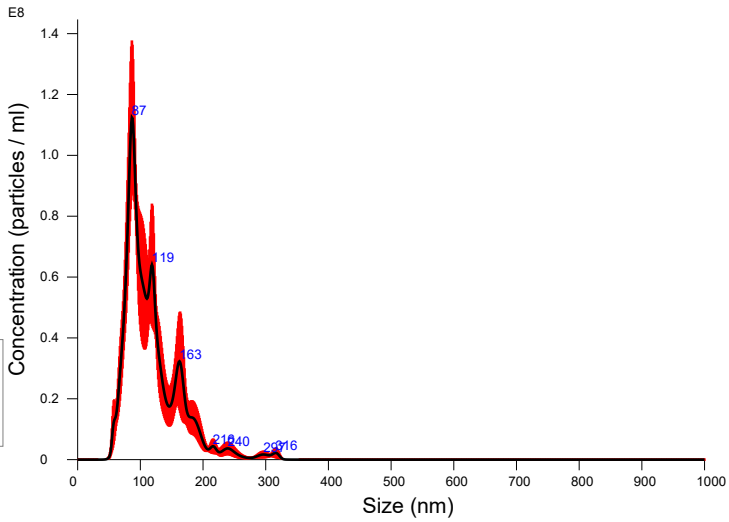
SOP Standard Measurement 11-25-53AM 12Mar2021.txt

NANOSIGHT

2021.03.12 Medium 2021-03-12 11-44-37



FTLA Concentration / Size graph for Experiment:
2021.03.12 Medium 2021-03-12 11-44-37



Averaged FTLA Concentration / Size for Experiment:
2021.03.12 Medium 2021-03-12 11-44-37
Error bars indicate + / -1 standard error of the mean

Included Files

2021.03.12 Medium 2021-03-12 11-47-25
2021.03.12 Medium 2021-03-12 11-48-34
2021.03.12 Medium 2021-03-12 11-49-43
2021.03.12 Medium 2021-03-12 11-50-52
2021.03.12 Medium 2021-03-12 11-52-01

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 11-44-05AM 12~
Time Captured:	11:44:37 12/03/2021
Operator:	Hanna
Pre-treatment:	1:50
Sample Name:	Medium
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	15
Slider Shutter:	1206
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	24.7 - 25.0 ºC
Viscosity:	(Water) 0.890 - 0.895 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 14.0 - 19.5 pix

Results

Stats: Merged Data

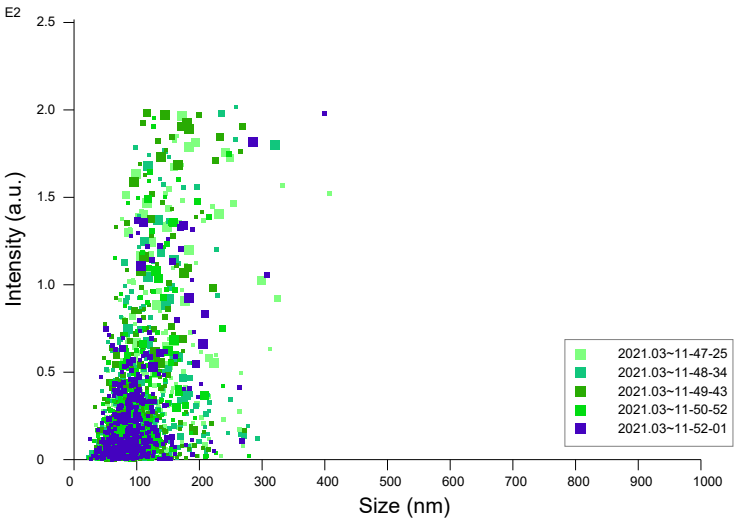
Mean:	117.3 nm
Mode:	86.9 nm
SD:	44.0 nm
D10:	76.4 nm
D50:	105.3 nm
D90:	173.8 nm

Stats: Mean +/- Standard Error

Mean:	117.0 +/- 2.2 nm
Mode:	89.0 +/- 5.6 nm
SD:	43.2 +/- 2.5 nm
D10:	76.4 +/- 1.7 nm
D50:	105.7 +/- 2.4 nm
D90:	176.5 +/- 6.4 nm
Concentration (Upgrade):	5.89e+09 +/- 3.09e+08 particles/ml
	7.7 +/- 0.6 particles/frame
	8.3 +/- 0.6 centres/frame

NANOSIGHT

2021.03.12 Medium 2021-03-12 11-44-37



Intensity / Size graph for Experiment:
2021.03.12 Medium 2021-03-12 11-44-37

Script Used: (Full Text):

SOP Standard Measurement 11-44-05AM 12Mar2021.txt

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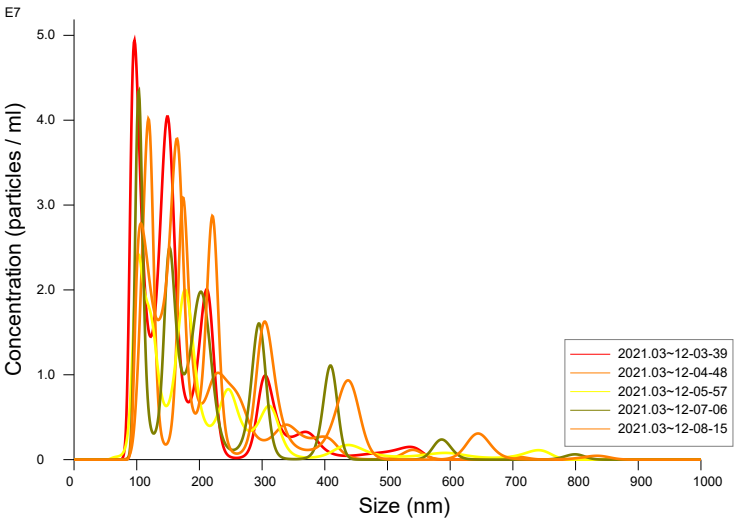
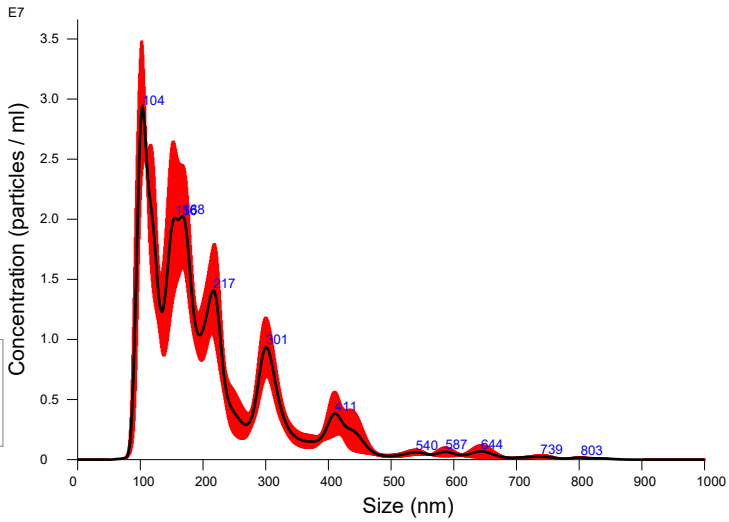
2021.03.12 MV ECA 10PE 2021-03-12 12-00-48



FTLA Concentration / Size graph for Experiment:
2021.03.12 MV ECA 10PE 2021-03-12 12-00-48



Averaged FTLA Concentration / Size for Experiment:
2021.03.12 MV ECA 10PE 2021-03-12 12-00-48
Error bars indicate + / -1 standard error of the mean

Included Files

2021.03.12 MV ECA 10PE 2021-03-12 12-03-39
2021.03.12 MV ECA 10PE 2021-03-12 12-04-48
2021.03.12 MV ECA 10PE 2021-03-12 12-05-57
2021.03.12 MV ECA 10PE 2021-03-12 12-07-06
2021.03.12 MV ECA 10PE 2021-03-12 12-08-15

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 12-00-20PM 12~
Time Captured:	12:00:48 12/03/2021
Operator:	Hanna
Pre-treatment:	1:10
Sample Name:	MV ECA 10PE
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	13
Slider Shutter:	1232
Slider Gain:	219
FPS	25.0
Number of Frames:	1498
Temperature:	25.1 - 25.3 ºC
Viscosity:	(Water) 0.882 - 0.887 cP
Dilution factor:	1 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 9.7 - 11.8 pix

Results

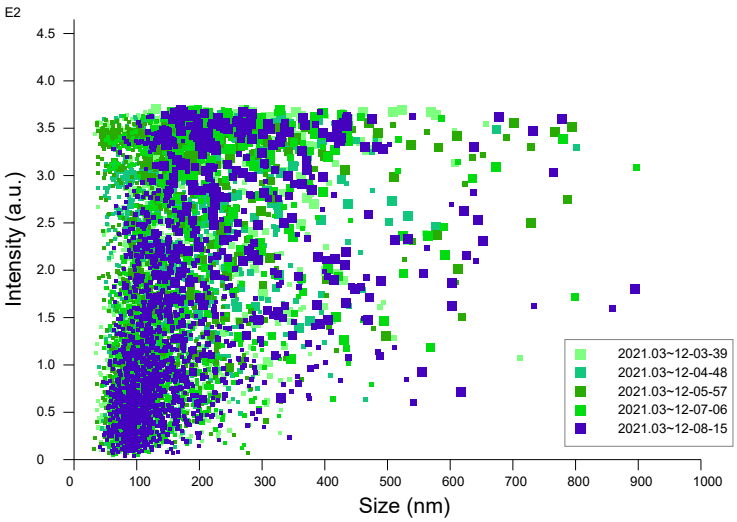
Stats: Merged Data

Mean:	213.2 nm
Mode:	103.3 nm
SD:	121.1 nm
D10:	104.0 nm
D50:	174.7 nm
D90:	384.6 nm

Stats: Mean +/- Standard Error

Mean:	212.9 +/- 9.1 nm
Mode:	117.0 +/- 12.3 nm
SD:	118.3 +/- 8.7 nm
D10:	105.3 +/- 3.1 nm
D50:	177.1 +/- 7.0 nm
D90:	379.5 +/- 20.4 nm
Concentration (Upgrade):	3.34e+09 +/- 3.03e+08 particles/ml
	34.7 +/- 2.3 particles/frame
	42.7 +/- 1.6 centres/frame

Concentration measurements may require some caution due to noise
See summary file for more info



Intensity / Size graph for Experiment:
2021.03.12 MV ECA 10PE 2021-03-12 12-00-48

Script Used: (Full Text):

SOP Standard Measurement 12-00-20PM 12Mar2021.txt

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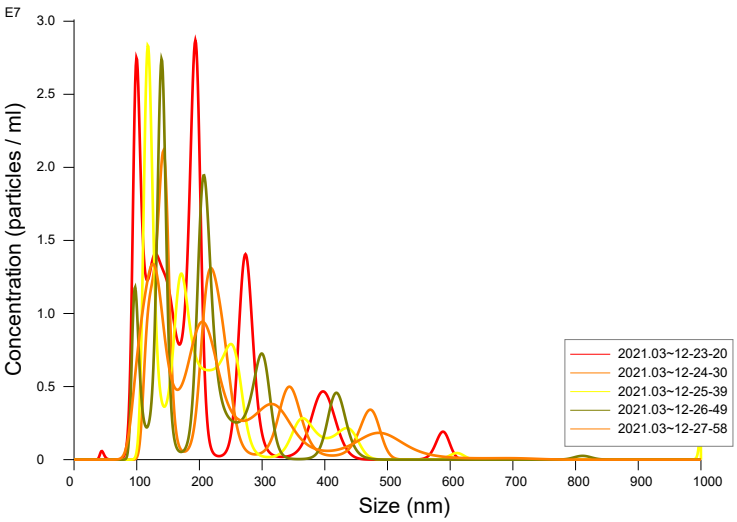
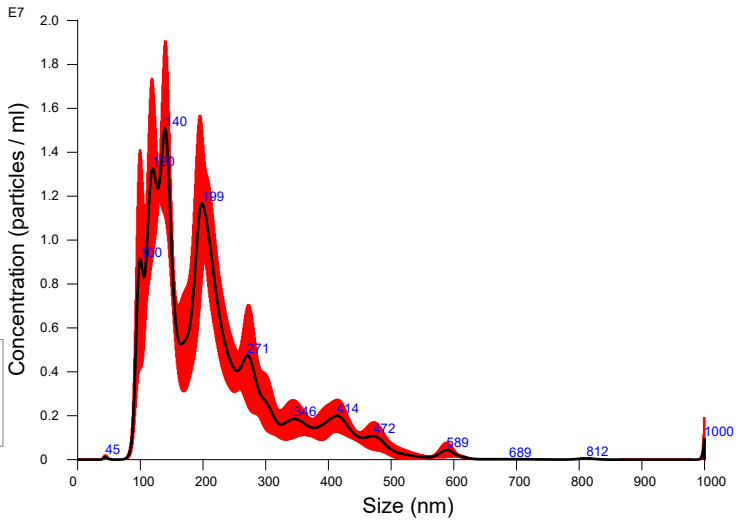
2021.03.12 MV ECA 150 2021-03-12 12-20-39



FTLA Concentration / Size graph for Experiment:
2021.03.12 MV ECA 150 2021-03-12 12-20-39



Averaged FTLA Concentration / Size for Experiment:
2021.03.12 MV ECA 150 2021-03-12 12-20-39
Error bars indicate + / -1 standard error of the mean

Included Files

2021.03.12 MV ECA 150 2021-03-12 12-23-20
2021.03.12 MV ECA 150 2021-03-12 12-24-30
2021.03.12 MV ECA 150 2021-03-12 12-25-39
2021.03.12 MV ECA 150 2021-03-12 12-26-49
2021.03.12 MV ECA 150 2021-03-12 12-27-58

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 12-20-24PM 12~
Time Captured:	12:20:39 12/03/2021
Operator:	Hanna
Pre-treatment:	1:10
Sample Name:	MV ECA 150
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	13
Slider Shutter:	1232
Slider Gain:	219
FPS	25.0
Number of Frames:	1498
Temperature:	25.3 - 25.5 ºC
Viscosity:	(Water) 0.879 - 0.882 cP
Dilution factor:	1 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 10.0 - 12.1 pix

Results

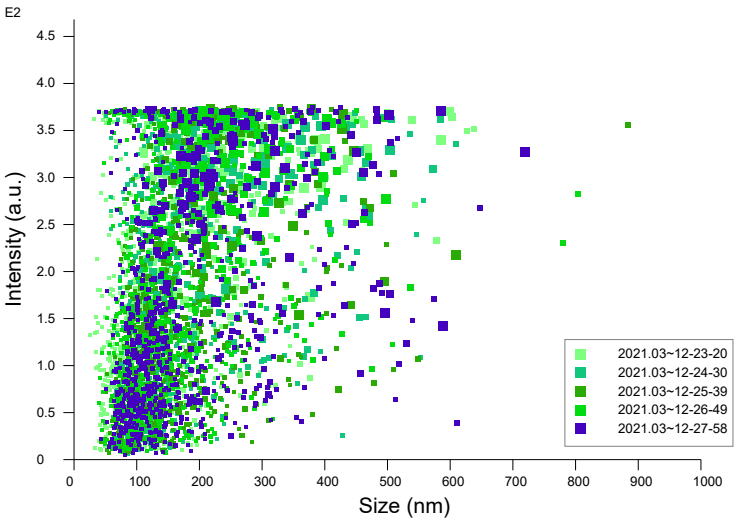
Stats: Merged Data

Mean:	216.4 nm
Mode:	139.5 nm
SD:	109.0 nm
D10:	112.9 nm
D50:	193.4 nm
D90:	379.5 nm

Stats: Mean +/- Standard Error

Mean:	217.8 +/- 5.3 nm
Mode:	143.9 +/- 13.2 nm
SD:	108.4 +/- 4.3 nm
D10:	114.6 +/- 4.0 nm
D50:	196.2 +/- 6.0 nm
D90:	379.7 +/- 15.5 nm
Concentration (Upgrade):	1.94e+09 +/- 1.54e+08 particles/ml
	20.1 +/- 1.8 particles/frame
	24.5 +/- 3.2 centres/frame

Concentration measurements may require some caution due to noise
See summary file for more info



Intensity / Size graph for Experiment:
2021.03.12 MV ECA 150 2021-03-12 12-20-39

Script Used: (Full Text):

SOP Standard Measurement 12-20-24PM 12Mar2021.txt

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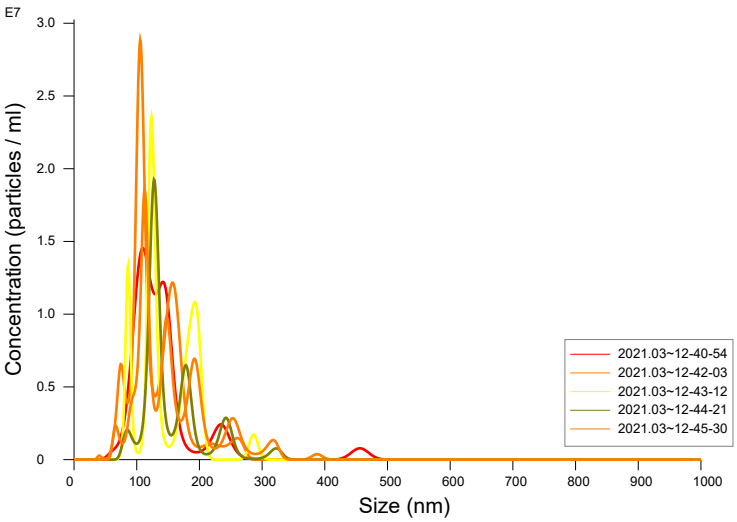
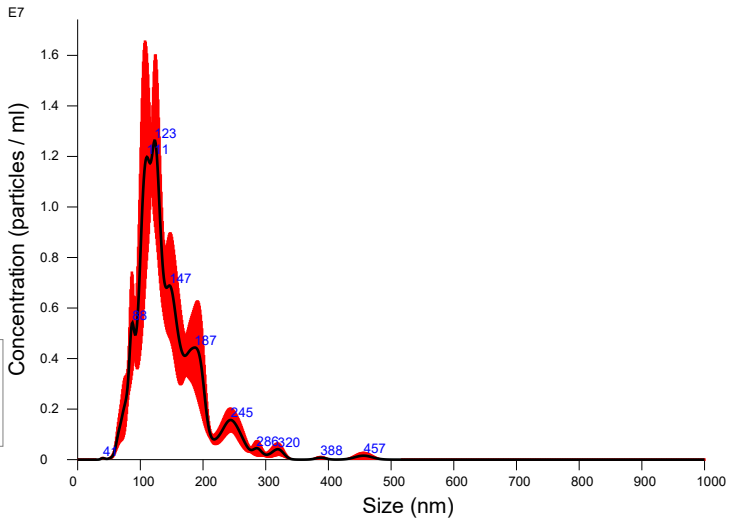
2021.03.12 MV Medium 2021-03-12 12-38-08



FTLA Concentration / Size graph for Experiment:
2021.03.12 MV Medium 2021-03-12 12-38-08



Averaged FTLA Concentration / Size for Experiment:
2021.03.12 MV Medium 2021-03-12 12-38-08
Error bars indicate + / -1 standard error of the mean

Included Files

2021.03.12 MV Medium 2021-03-12 12-40-54
2021.03.12 MV Medium 2021-03-12 12-42-03
2021.03.12 MV Medium 2021-03-12 12-43-12
2021.03.12 MV Medium 2021-03-12 12-44-21
2021.03.12 MV Medium 2021-03-12 12-45-30

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 12-37-51PM 12~
Time Captured:	12:38:08 12/03/2021
Operator:	Hanna
Pre-treatment:	1:10
Sample Name:	MV Medium
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	13
Slider Shutter:	1232
Slider Gain:	219
FPS	25.0
Number of Frames:	1498
Temperature:	25.1 - 25.2 ºC
Viscosity:	(Water) 0.884 - 0.887 cP
Dilution factor:	1 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 11.6 - 23.1 pix

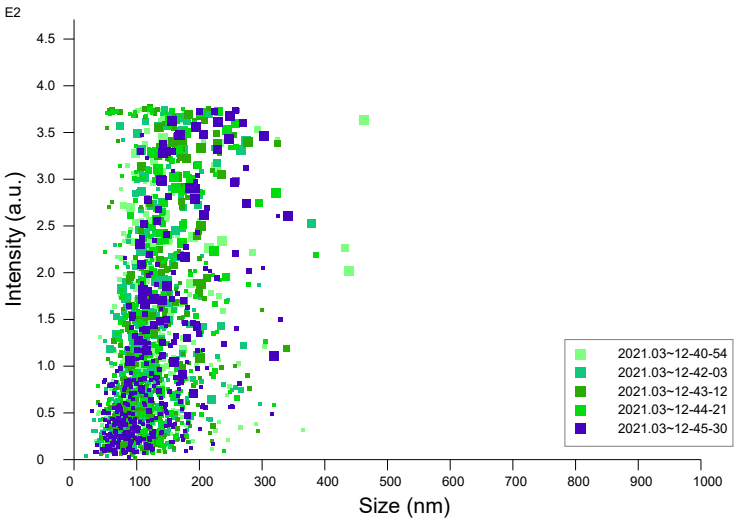
Results

Stats: Merged Data

Mean:	145.3 nm
Mode:	123.0 nm
SD:	54.6 nm
D10:	93.4 nm
D50:	130.6 nm
D90:	208.5 nm

Stats: Mean +/- Standard Error

Mean:	146.5 +/- 4.4 nm
Mode:	115.8 +/- 4.2 nm
SD:	53.2 +/- 3.5 nm
D10:	96.7 +/- 5.0 nm
D50:	130.4 +/- 4.6 nm
D90:	216.1 +/- 12.6 nm
Concentration (Upgrade):	9.80e+08 +/- 8.25e+07 particles/ml
	8.6 +/- 0.6 particles/frame
	9.0 +/- 0.4 centres/frame



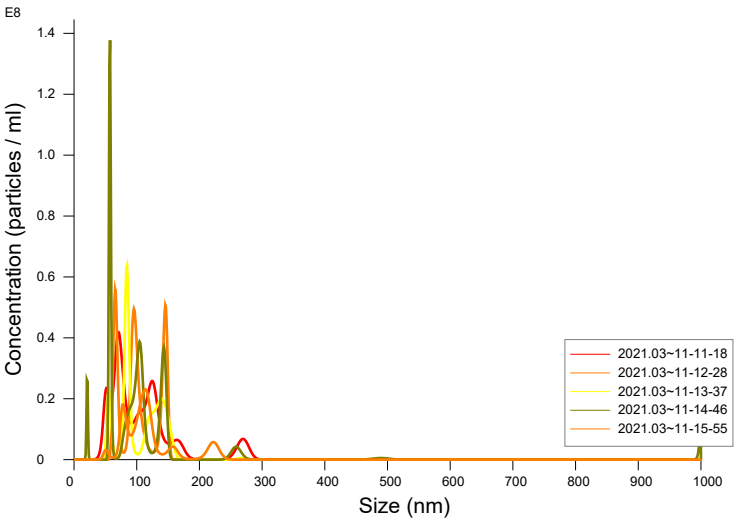
Intensity / Size graph for Experiment:
2021.03.12 MV Medium 2021-03-12 12-38-08

Script Used: (Full Text):

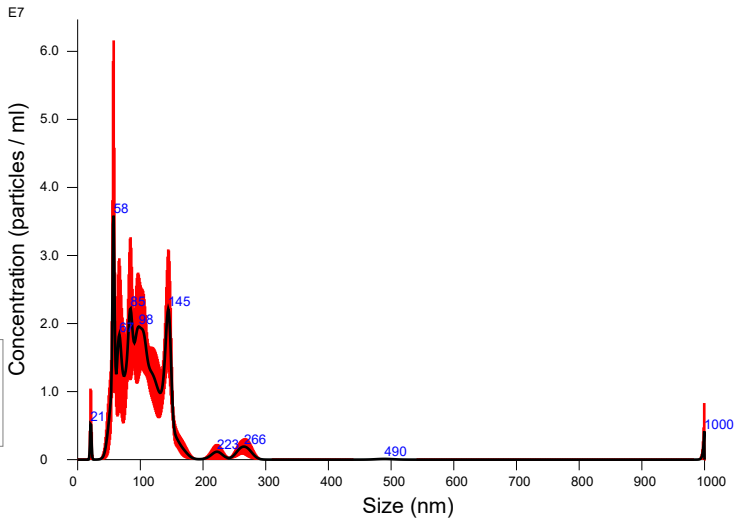
SOP Standard Measurement 12-37-51PM 12Mar2021.txt

NANOSIGHT

2021.03.12 PBS 2021-03-12 11-08-37



FTLA Concentration / Size graph for Experiment:
2021.03.12 PBS 2021-03-12 11-08-37



Averaged FTLA Concentration / Size for Experiment:
2021.03.12 PBS 2021-03-12 11-08-37
Error bars indicate + / -1 standard error of the mean

Included Files

2021.03.12 PBS 2021-03-12 11-11-18
2021.03.12 PBS 2021-03-12 11-12-28
2021.03.12 PBS 2021-03-12 11-13-37
2021.03.12 PBS 2021-03-12 11-14-46
2021.03.12 PBS 2021-03-12 11-15-55

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 10-51-38AM 12~
Time Captured:	11:08:37 12/03/2021
Operator:	Hanna
Pre-treatment:	
Sample Name:	PBS
Diluent:	
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	23.9 - 24.1 ºC
Viscosity:	(Water) 0.907 - 0.911 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 14.2 - 30.5 pix

Results

Stats: Merged Data

Mean:	111.8 nm
Mode:	57.1 nm
SD:	80.0 nm
D10:	58.0 nm
D50:	100.1 nm
D90:	149.4 nm

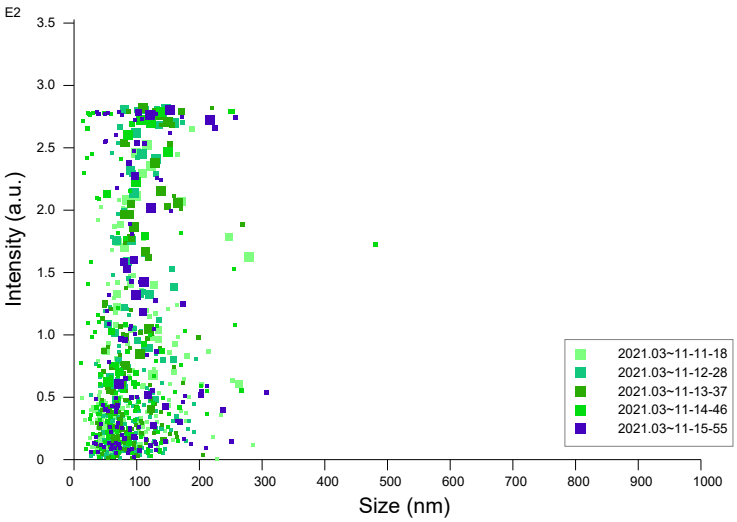
Stats: Mean +/- Standard Error

Mean:	112.2 +/- 4.1 nm
Mode:	78.3 +/- 9.8 nm
SD:	61.8 +/- 21.5 nm
D10:	62.5 +/- 3.9 nm
D50:	99.3 +/- 3.1 nm
D90:	163.6 +/- 12.5 nm
Concentration (Upgrade):	1.82e+09 +/- 2.15e+08 particles/ml
	3.2 +/- 0.3 particles/frame
	4.8 +/- 1.1 centres/frame

Concentration measurements may be unreliable
See summary file for more info

NANOSIGHT

2021.03.12 PBS 2021-03-12 11-08-37



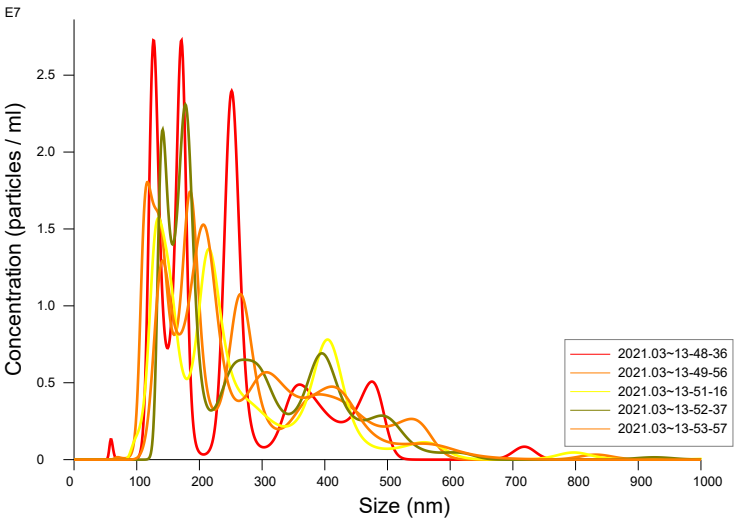
Intensity / Size graph for Experiment:
2021.03.12 PBS 2021-03-12 11-08-37

Script Used: (Full Text):

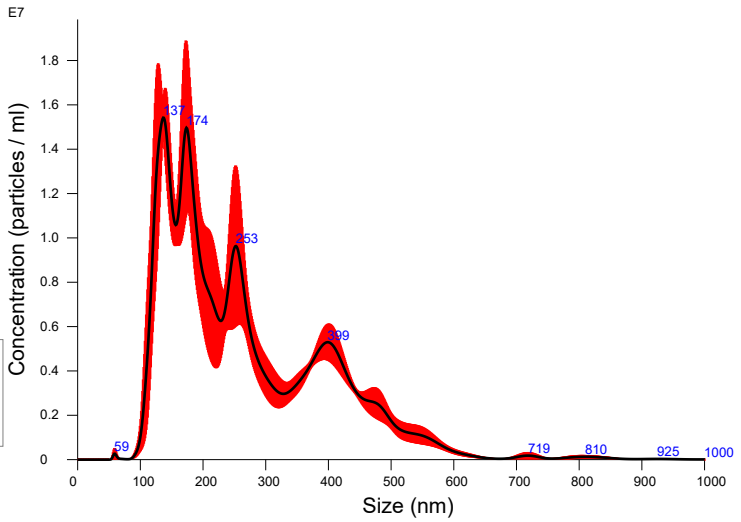
SOP Standard Measurement 10-51-38AM 12Mar2021.txt

NANOSIGHT

2021.03.25 MV ECA159 2021-03-25 13-45-43



FTLA Concentration / Size graph for Experiment:
2021.03.25 MV ECA159 2021-03-25 13-45-43



Averaged FTLA Concentration / Size for Experiment:
2021.03.25 MV ECA159 2021-03-25 13-45-43
Error bars indicate + / -1 standard error of the mean

Included Files

2021.03.25 MV ECA159 2021-03-25 13-48-36
2021.03.25 MV ECA159 2021-03-25 13-49-56
2021.03.25 MV ECA159 2021-03-25 13-51-16
2021.03.25 MV ECA159 2021-03-25 13-52-37
2021.03.25 MV ECA159 2021-03-25 13-53-57

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 01-45-15PM 25~
Time Captured:	13:45:43 25/03/2021
Operator:	Hanna
Pre-treatment:	1:10
Sample Name:	MV ECA 159
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	11
Slider Shutter:	890
Slider Gain:	146
FPS	25.0
Number of Frames:	1498
Temperature:	24.6 - 24.8 ºC
Viscosity:	(Water) 0.893 - 0.897 cP
Dilution factor:	1 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 9.4 - 11.8 pix

Results

Stats: Merged Data

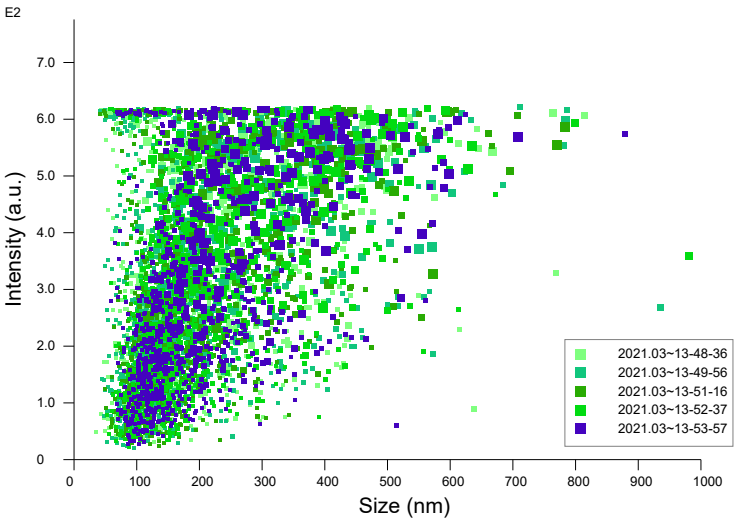
Mean:	265.4 nm
Mode:	136.9 nm
SD:	129.6 nm
D10:	132.8 nm
D50:	230.4 nm
D90:	447.9 nm

Stats: Mean +/- Standard Error

Mean:	265.6 +/- 4.3 nm
Mode:	152.5 +/- 16.9 nm
SD:	129.0 +/- 3.4 nm
D10:	133.7 +/- 4.7 nm
D50:	228.9 +/- 5.5 nm
D90:	448.6 +/- 5.9 nm
Concentration (Upgrade):	2.63e+09 +/- 7.18e+07 particles/ml
	26.2 +/- 0.6 particles/frame
	29.0 +/- 0.7 centres/frame

NANOSIGHT

2021.03.25 MV ECA159 2021-03-25 13-45-43



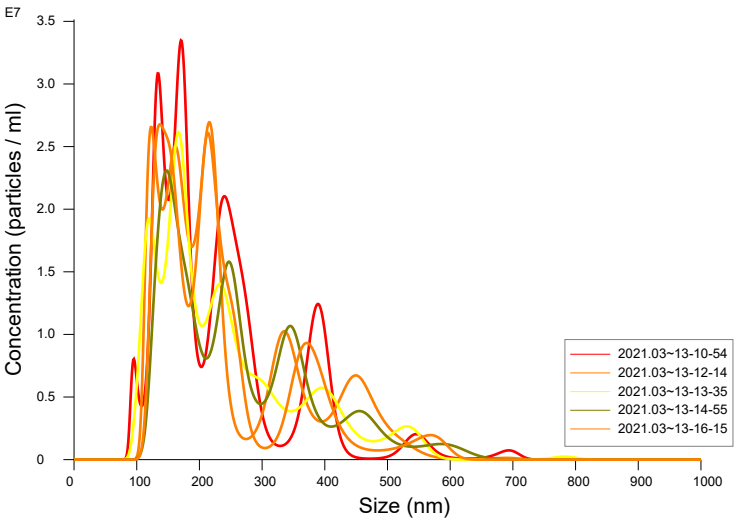
Intensity / Size graph for Experiment:
2021.03.25 MV ECA159 2021-03-25 13-45-43

Script Used: (Full Text):

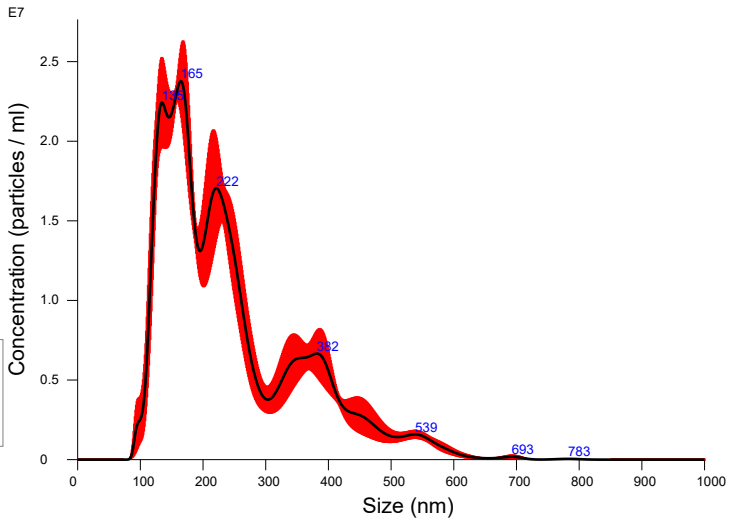
SOP Standard Measurement 01-45-15PM 25Mar2021.txt

NANOSIGHT

2021.03.25 MV ECV42 2021-03-25 13-08-09



FTLA Concentration / Size graph for Experiment:
2021.03.25 MV ECV42 2021-03-25 13-08-09



Averaged FTLA Concentration / Size for Experiment:
2021.03.25 MV ECV42 2021-03-25 13-08-09
Error bars indicate + / -1 standard error of the mean

Included Files

2021.03.25 MV ECV42 2021-03-25 13-10-54
2021.03.25 MV ECV42 2021-03-25 13-12-14
2021.03.25 MV ECV42 2021-03-25 13-13-35
2021.03.25 MV ECV42 2021-03-25 13-14-55
2021.03.25 MV ECV42 2021-03-25 13-16-15

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 01-07-29PM 25~
Time Captured:	13:08:09 25/03/2021
Operator:	Hanna
Pre-treatment:	1:10
Sample Name:	MV ECV 42
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	11
Slider Shutter:	890
Slider Gain:	146
FPS	25.0
Number of Frames:	1498
Temperature:	24.3 - 24.4 ºC
Viscosity:	(Water) 0.901 - 0.903 cP
Dilution factor:	1 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 9.5 - 10.9 pix

Results

Stats: Merged Data

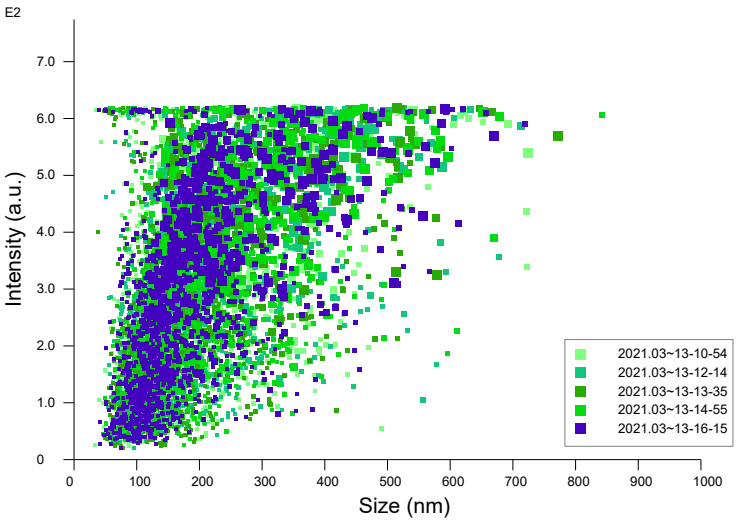
Mean:	244.0 nm
Mode:	164.6 nm
SD:	113.2 nm
D10:	131.5 nm
D50:	212.9 nm
D90:	408.1 nm

Stats: Mean +/- Standard Error

Mean:	244.5 +/- 5.0 nm
Mode:	167.6 +/- 13.6 nm
SD:	112.8 +/- 1.9 nm
D10:	131.4 +/- 3.0 nm
D50:	214.6 +/- 6.1 nm
D90:	415.2 +/- 10.6 nm
Concentration (Upgrade):	3.89e+09 +/- 1.37e+08 particles/ml
	37.4 +/- 1.1 particles/frame
	39.1 +/- 1.0 centres/frame

NANOSIGHT

2021.03.25 MV ECV42 2021-03-25 13-08-09



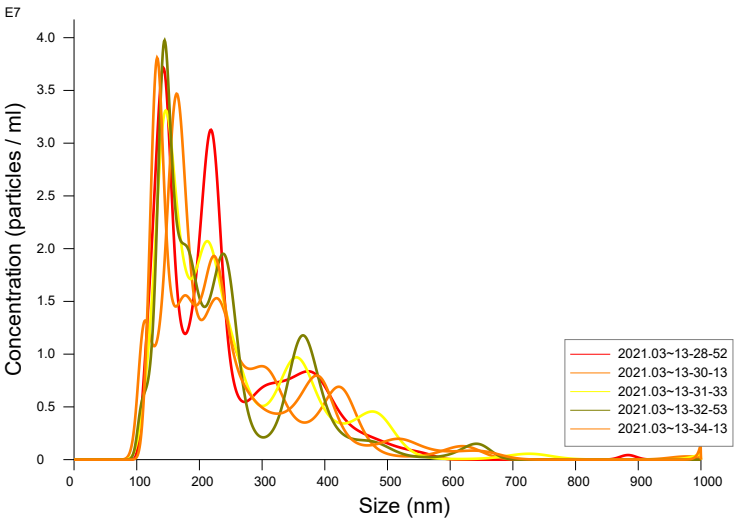
Intensity / Size graph for Experiment:
2021.03.25 MV ECV42 2021-03-25 13-08-09

Script Used: (Full Text):

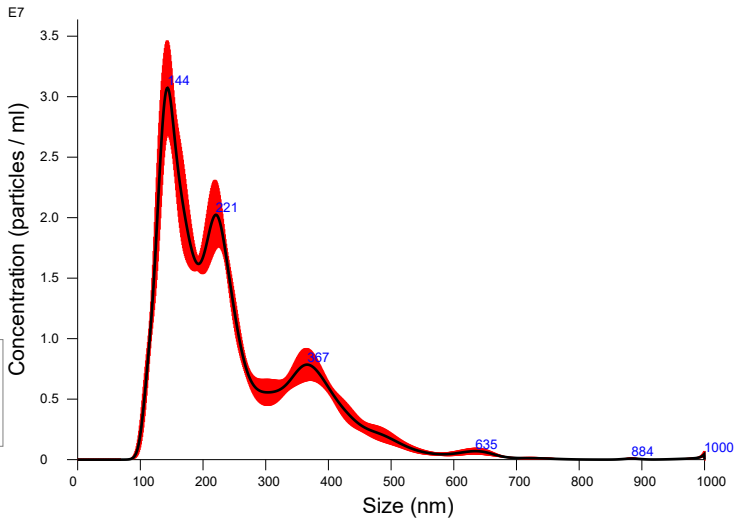
SOP Standard Measurement 01-07-29PM 25Mar2021.txt

NANOSIGHT

2021.03.25 MV ECV150 2021-03-25 13-26-12



FTLA Concentration / Size graph for Experiment:
2021.03.25 MV ECV150 2021-03-25 13-26-12



Averaged FTLA Concentration / Size for Experiment:
2021.03.25 MV ECV150 2021-03-25 13-26-12
Error bars indicate + / -1 standard error of the mean

Included Files

2021.03.25 MV ECV150 2021-03-25 13-28-52
2021.03.25 MV ECV150 2021-03-25 13-30-13
2021.03.25 MV ECV150 2021-03-25 13-31-33
2021.03.25 MV ECV150 2021-03-25 13-32-53
2021.03.25 MV ECV150 2021-03-25 13-34-13

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 01-25-49PM 25~
Time Captured:	13:26:12 25/03/2021
Operator:	Hanna
Pre-treatment:	1:10
Sample Name:	MV ECV 150
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	11
Slider Shutter:	890
Slider Gain:	146
FPS	25.0
Number of Frames:	1498
Temperature:	24.2 - 24.4 ºC
Viscosity:	(Water) 0.900 - 0.904 cP
Dilution factor:	1 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 10.2 - 10.9 pix

Results

Stats: Merged Data

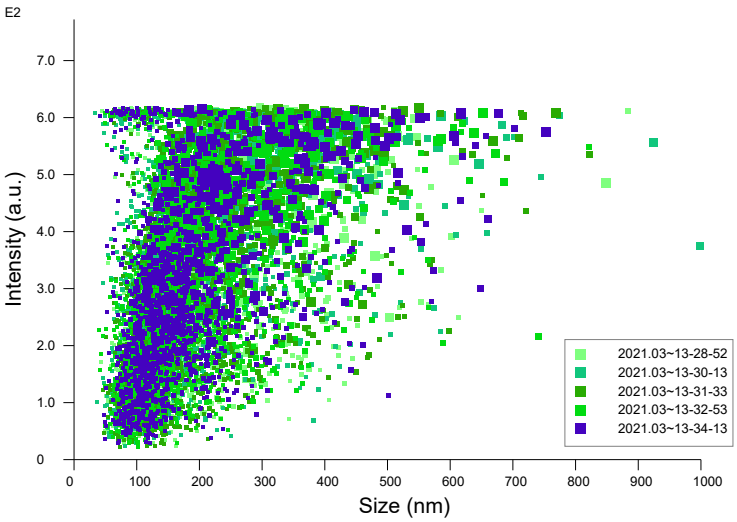
Mean:	246.1 nm
Mode:	143.3 nm
SD:	118.2 nm
D10:	133.9 nm
D50:	213.4 nm
D90:	407.3 nm

Stats: Mean +/- Standard Error

Mean:	246.1 +/- 2.2 nm
Mode:	145.9 +/- 5.0 nm
SD:	118.0 +/- 3.5 nm
D10:	134.3 +/- 1.4 nm
D50:	212.9 +/- 1.1 nm
D90:	409.0 +/- 7.0 nm
Concentration (Upgrade):	4.32e+09 +/- 9.87e+07 particles/ml
	44.0 +/- 0.8 particles/frame
	49.2 +/- 1.2 centres/frame

NANOSIGHT

2021.03.25 MV ECV150 2021-03-25 13-26-12



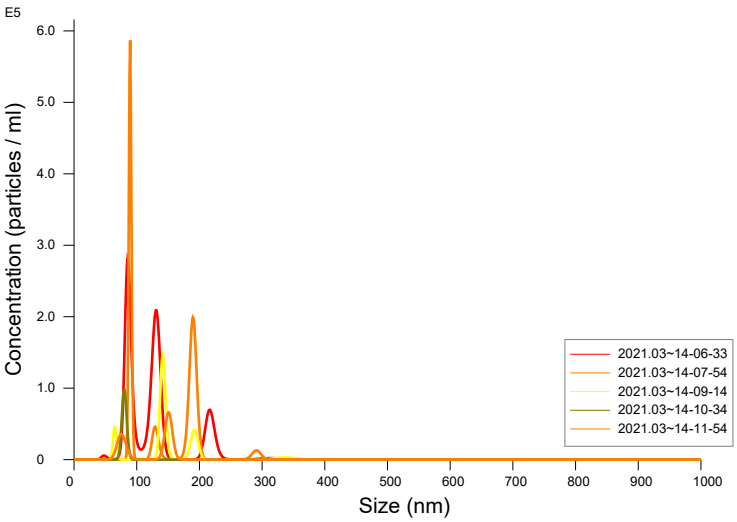
Intensity / Size graph for Experiment:
2021.03.25 MV ECV150 2021-03-25 13-26-12

Script Used: (Full Text):

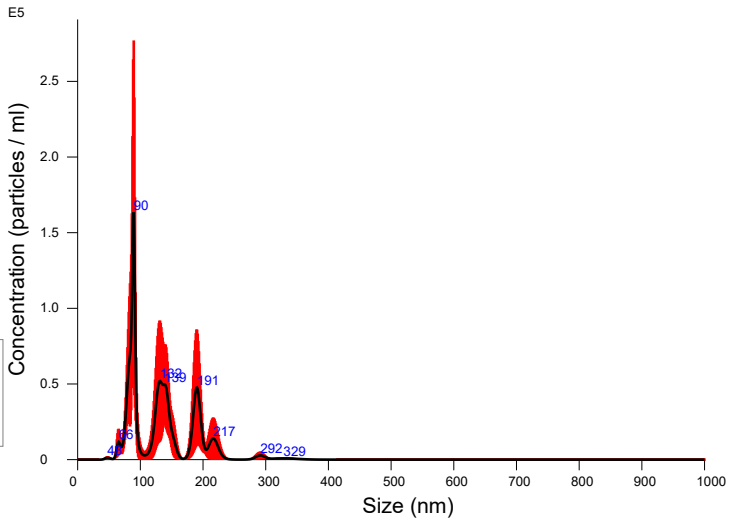
SOP Standard Measurement 01-25-49PM 25Mar2021.txt

NANOSIGHT

2021.03.25 PBS 2021-03-25 14-03-50



FTLA Concentration / Size graph for Experiment:
2021.03.25 PBS 2021-03-25 14-03-50



Averaged FTLA Concentration / Size for Experiment:
2021.03.25 PBS 2021-03-25 14-03-50
Error bars indicate + / -1 standard error of the mean

Included Files

2021.03.25 PBS 2021-03-25 14-06-33
2021.03.25 PBS 2021-03-25 14-07-54
2021.03.25 PBS 2021-03-25 14-09-14
2021.03.25 PBS 2021-03-25 14-10-34
2021.03.25 PBS 2021-03-25 14-11-54

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 02-02-38PM 25~
Time Captured:	14:03:50 25/03/2021
Operator:	Hanna
Pre-treatment:	
Sample Name:	PBs
Diluent:	
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	24.9 - 25.1 ºC
Viscosity:	(Water) 0.887 - 0.892 cP
Dilution factor:	Dilution not recorded
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 14.2 pix

Results

Stats: Merged Data

Mean:	132.7 nm
Mode:	89.1 nm
SD:	52.0 nm
D10:	81.5 nm
D50:	128.5 nm
D90:	198.0 nm

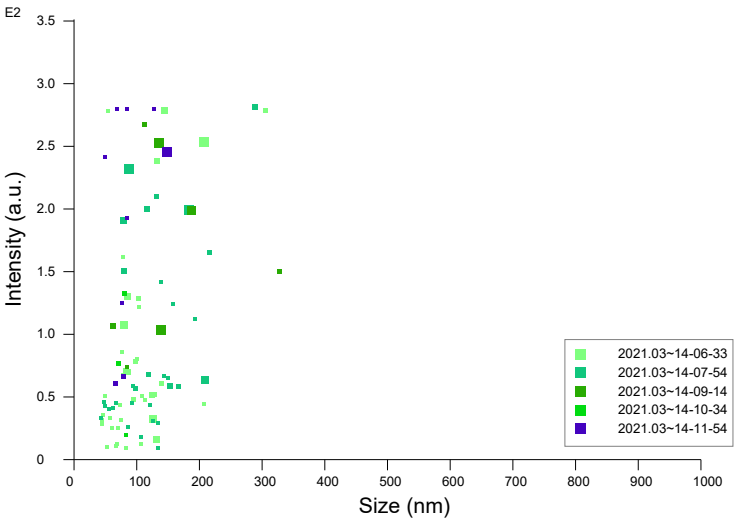
Stats: Mean +/- Standard Error

Mean:	125.0 +/- 12.7 nm
Mode:	109.5 +/- 15.1 nm
SD:	39.9 +/- 9.6 nm
D10:	76.9 +/- 3.7 nm
D50:	124.6 +/- 11.8 nm
D90:	169.8 +/- 23.2 nm
Concentration (Upgrade):	4.27e+06 +/- 1.60e+06 particles/ml
	0.4 +/- 0.1 particles/frame
	0.4 +/- 0.1 centres/frame

Concentration measurements may be unreliable
See summary file for more info

NANOSIGHT

2021.03.25 PBS 2021-03-25 14-03-50



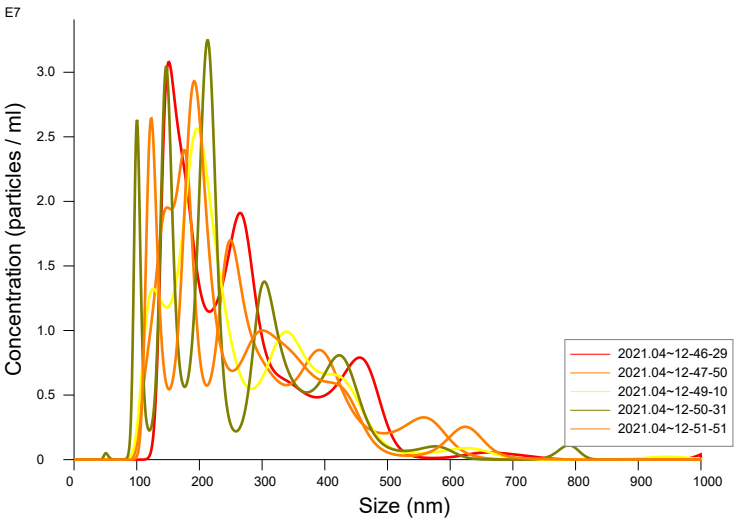
Intensity / Size graph for Experiment:
2021.03.25 PBS 2021-03-25 14-03-50

Script Used: (Full Text):

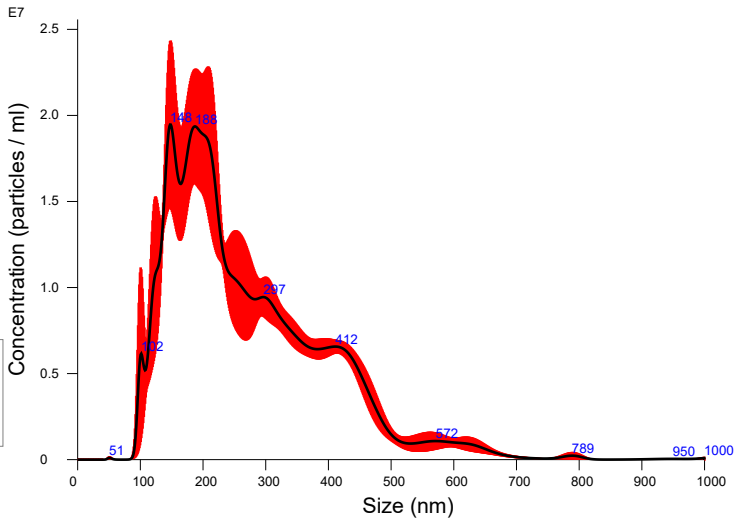
SOP Standard Measurement 02-02-38PM 25Mar2021.txt

NANOSIGHT

2021.04.14 MV ECA 1PE 2021-04-14 12-43-45



FTLA Concentration / Size graph for Experiment:
2021.04.14 MV ECA 1PE 2021-04-14 12-43-45



Averaged FTLA Concentration / Size for Experiment:
2021.04.14 MV ECA 1PE 2021-04-14 12-43-45
Error bars indicate + / -1 standard error of the mean

Included Files

2021.04.14 MV ECA 1PE 2021-04-14 12-46-29
2021.04.14 MV ECA 1PE 2021-04-14 12-47-50
2021.04.14 MV ECA 1PE 2021-04-14 12-49-10
2021.04.14 MV ECA 1PE 2021-04-14 12-50-31
2021.04.14 MV ECA 1PE 2021-04-14 12-51-51

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 12-43-41PM 14A~
Time Captured:	12:43:45 14/04/2021
Operator:	Hanna
Pre-treatment:	1:10
Sample Name:	MV ECA 1PE
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	11
Slider Shutter:	890
Slider Gain:	146
FPS	25.0
Number of Frames:	1498
Temperature:	24.9 - 25.1 ºC
Viscosity:	(Water) 0.887 - 0.891 cP
Dilution factor:	1 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 9.9 - 12.4 pix

Results

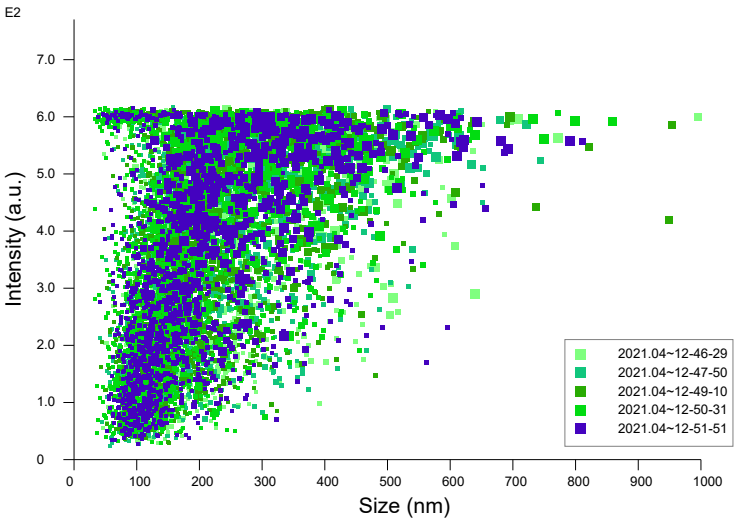
Stats: Merged Data

Mean:	269.0 nm
Mode:	148.0 nm
SD:	124.0 nm
D10:	140.1 nm
D50:	234.3 nm
D90:	437.4 nm

Stats: Mean +/- Standard Error

Mean:	268.9 +/- 2.2 nm
Mode:	185.6 +/- 10.5 nm
SD:	124.0 +/- 1.7 nm
D10:	138.1 +/- 3.9 nm
D50:	234.2 +/- 6.0 nm
D90:	438.4 +/- 5.7 nm
Concentration (Upgrade):	4.09e+09 +/- 5.54e+07 particles/ml
	43.7 +/- 0.5 particles/frame
	52.5 +/- 0.7 centres/frame

Concentration measurements may require some caution due to noise
See summary file for more info



Intensity / Size graph for Experiment:
2021.04.14 MV ECA 1PE 2021-04-14 12-43-45

Script Used: (Full Text):

SOP Standard Measurement 12-43-41PM 14Apr2021.txt

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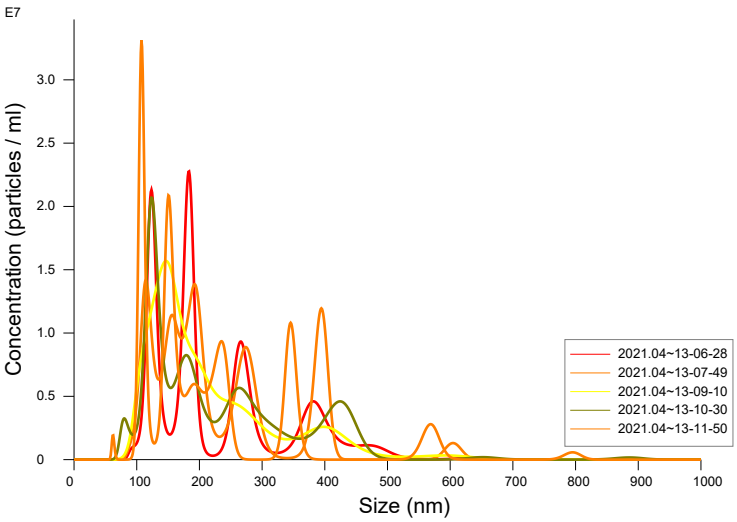
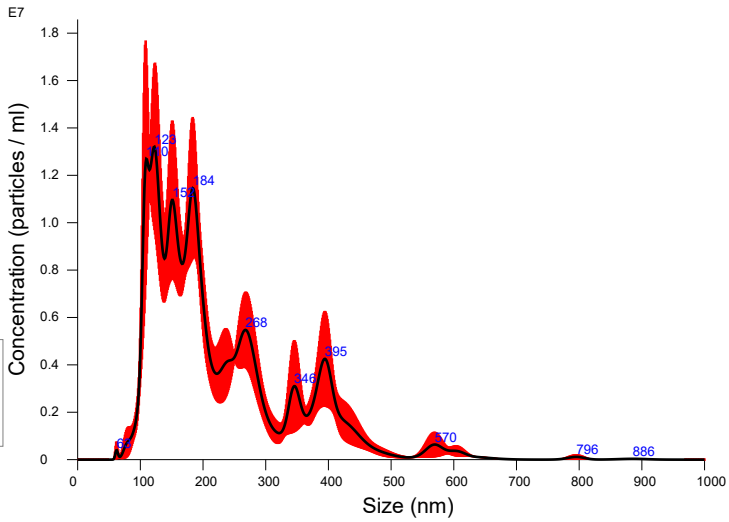
2021.04.14 MV ECA 18PE 2021-04-14 13-03-49



FTLA Concentration / Size graph for Experiment:
2021.04.14 MV ECA 18PE 2021-04-14 13-03-49



Averaged FTLA Concentration / Size for Experiment:
2021.04.14 MV ECA 18PE 2021-04-14 13-03-49
Error bars indicate + / -1 standard error of the mean

Included Files

2021.04.14 MV ECA 18PE 2021-04-14 13-06-28
2021.04.14 MV ECA 18PE 2021-04-14 13-07-49
2021.04.14 MV ECA 18PE 2021-04-14 13-09-10
2021.04.14 MV ECA 18PE 2021-04-14 13-10-30
2021.04.14 MV ECA 18PE 2021-04-14 13-11-50

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 01-03-40PM 14A~
Time Captured:	13:03:49 14/04/2021
Operator:	Hanna
Pre-treatment:	1:10
Sample Name:	MV ECA 18PE
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	11
Slider Shutter:	890
Slider Gain:	146
FPS	25.0
Number of Frames:	1498
Temperature:	25.3 - 25.5 ºC
Viscosity:	(Water) 0.879 - 0.882 cP
Dilution factor:	1 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 11.1 - 13.2 pix

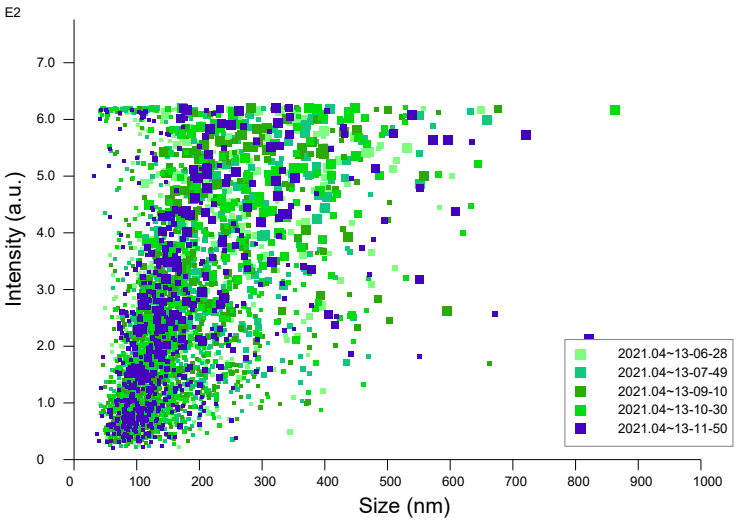
Results

Stats: Merged Data

Mean:	226.0 nm
Mode:	122.2 nm
SD:	116.1 nm
D10:	114.3 nm
D50:	187.5 nm
D90:	395.5 nm

Stats: Mean +/- Standard Error

Mean:	226.1 +/- 3.6 nm
Mode:	135.2 +/- 13.7 nm
SD:	115.7 +/- 4.8 nm
D10:	115.7 +/- 2.4 nm
D50:	185.9 +/- 4.0 nm
D90:	390.0 +/- 10.7 nm
Concentration (Upgrade):	1.89e+09 +/- 6.10e+07 particles/ml
	17.2 +/- 0.5 particles/frame
	18.4 +/- 0.5 centres/frame



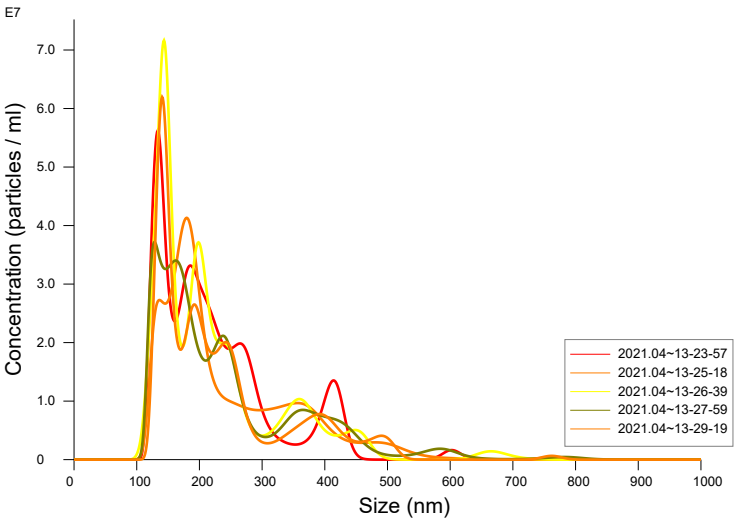
Intensity / Size graph for Experiment:
2021.04.14 MV ECA 18PE 2021-04-14 13-03-49

Script Used: (Full Text):

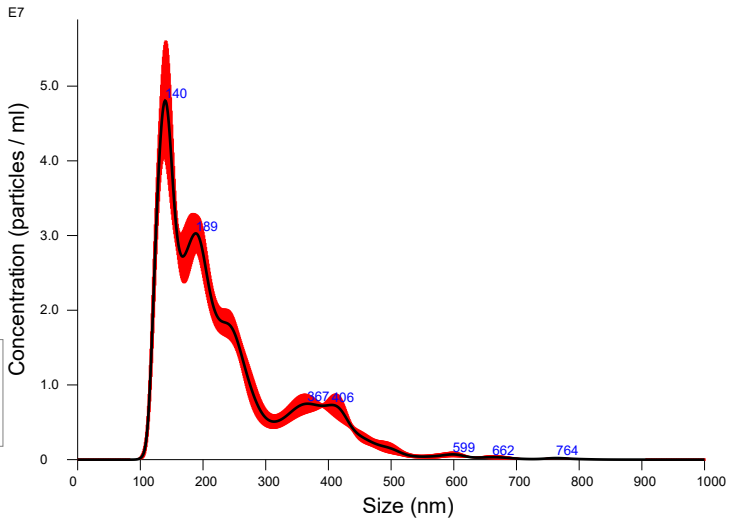
SOP Standard Measurement 01-03-40PM 14Apr2021.txt

NANOSIGHT

2021.04.14 MV ECV 159 2021-04-14 13-21-12



FTLA Concentration / Size graph for Experiment:
2021.04.14 MV ECV 159 2021-04-14 13-21-12



Averaged FTLA Concentration / Size for Experiment:
2021.04.14 MV ECV 159 2021-04-14 13-21-12
Error bars indicate + / -1 standard error of the mean

Included Files

2021.04.14 MV ECV 159 2021-04-14 13-23-57
2021.04.14 MV ECV 159 2021-04-14 13-25-18
2021.04.14 MV ECV 159 2021-04-14 13-26-39
2021.04.14 MV ECV 159 2021-04-14 13-27-59
2021.04.14 MV ECV 159 2021-04-14 13-29-19

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 01-20-25PM 14A~
Time Captured:	13:21:12 14/04/2021
Operator:	Hanna
Pre-treatment:	1:10
Sample Name:	MV ECV 159
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	11
Slider Shutter:	890
Slider Gain:	146
FPS	25.0
Number of Frames:	1498
Temperature:	25.6 - 25.8 ºC
Viscosity:	(Water) 0.873 - 0.876 cP
Dilution factor:	1 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 10.2 - 10.8 pix

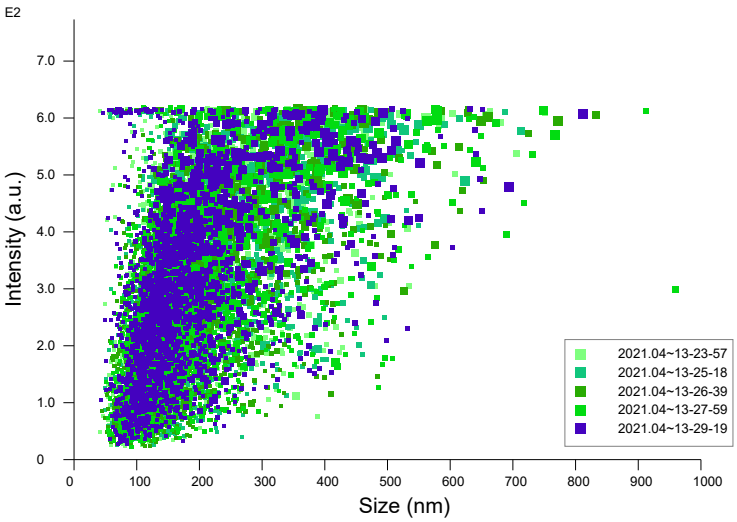
Results

Stats: Merged Data

Mean:	232.0 nm
Mode:	139.6 nm
SD:	106.4 nm
D10:	133.2 nm
D50:	198.2 nm
D90:	394.7 nm

Stats: Mean +/- Standard Error

Mean:	232.4 +/- 3.9 nm
Mode:	145.1 +/- 9.0 nm
SD:	106.1 +/- 4.2 nm
D10:	133.6 +/- 1.5 nm
D50:	198.6 +/- 1.4 nm
D90:	394.6 +/- 6.0 nm
Concentration (Upgrade):	5.51e+09 +/- 2.13e+08 particles/ml
	53.0 +/- 1.9 particles/frame
	55.0 +/- 1.5 centres/frame



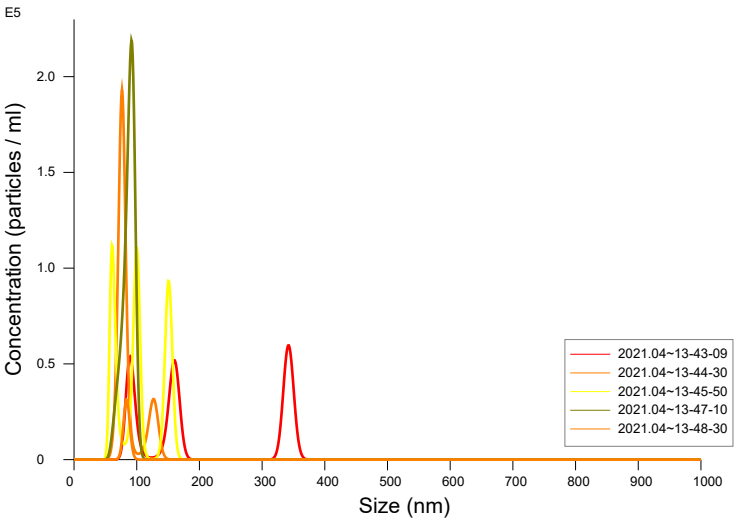
Intensity / Size graph for Experiment:
2021.04.14 MV ECV 159 2021-04-14 13-21-12

Script Used: (Full Text):

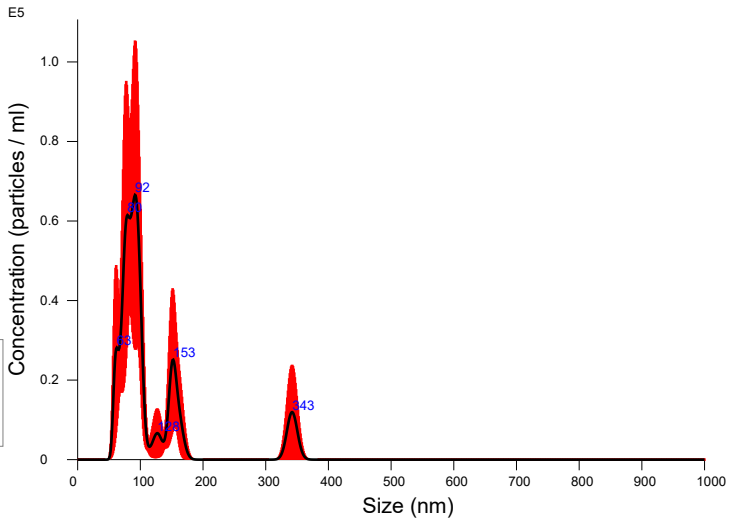
SOP Standard Measurement 01-20-25PM 14Apr2021.txt

NANOSIGHT

2021.04.14 PBS 2021-04-14 13-40-27



FTLA Concentration / Size graph for Experiment:
2021.04.14 PBS 2021-04-14 13-40-27



Averaged FTLA Concentration / Size for Experiment:
2021.04.14 PBS 2021-04-14 13-40-27
Error bars indicate + / -1 standard error of the mean

Included Files

2021.04.14 PBS 2021-04-14 13-43-09
2021.04.14 PBS 2021-04-14 13-44-30
2021.04.14 PBS 2021-04-14 13-45-50
2021.04.14 PBS 2021-04-14 13-47-10
2021.04.14 PBS 2021-04-14 13-48-30

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 01-40-07PM 14A~
Time Captured:	13:40:27 14/04/2021
Operator:	Hanna
Pre-treatment:	
Sample Name:	PBS
Diluent:	
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	25.9 - 26.1 ºC
Viscosity:	(Water) 0.867 - 0.870 cP
Dilution factor:	Dilution not recorded
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 14.2 pix

Results

Stats: Merged Data

Mean:	116.0 nm
Mode:	91.7 nm
SD:	71.1 nm
D10:	67.9 nm
D50:	91.3 nm
D90:	163.6 nm

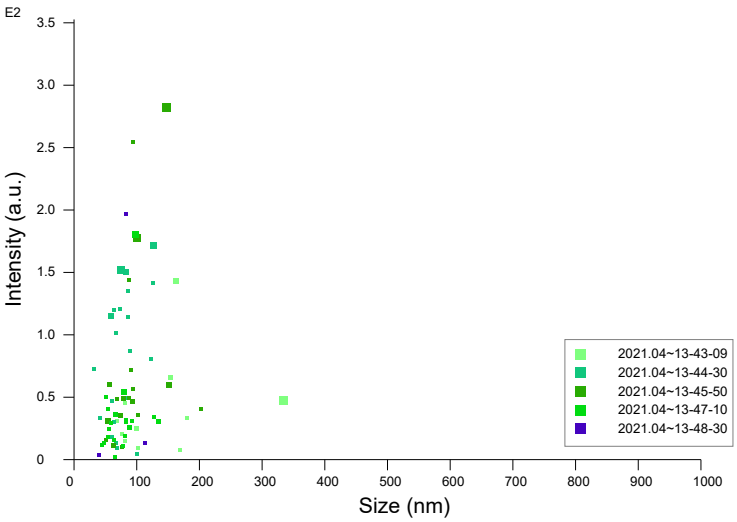
Stats: Mean +/- Standard Error

Mean:	113.3 +/- 23.4 nm
Mode:	131.0 +/- 53.0 nm
SD:	35.6 +/- 18.7 nm
D10:	73.3 +/- 4.5 nm
D50:	102.4 +/- 15.2 nm
D90:	162.9 +/- 47.4 nm
Concentration (Upgrade):	3.24e+06 +/- 7.45e+05 particles/ml
	0.2 +/- 0.1 particles/frame
	0.2 +/- 0.1 centres/frame

Concentration measurements may be unreliable
See summary file for more info

NANOSIGHT

2021.04.14 PBS 2021-04-14 13-40-27



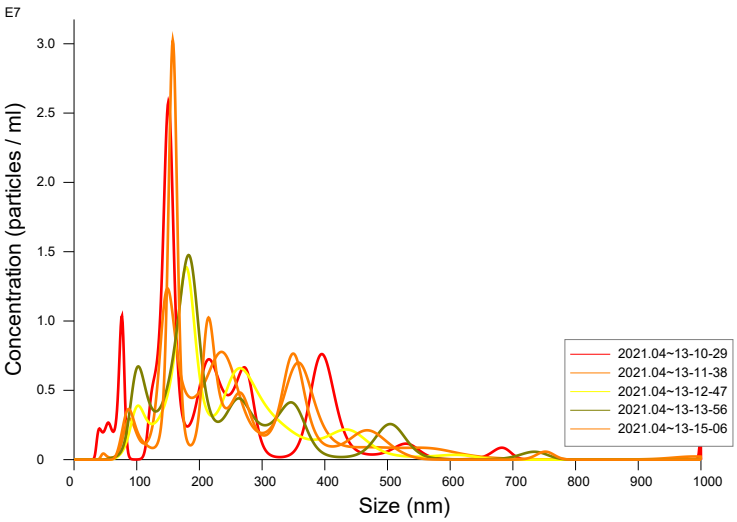
Intensity / Size graph for Experiment:
2021.04.14 PBS 2021-04-14 13-40-27

Script Used: (Full Text):

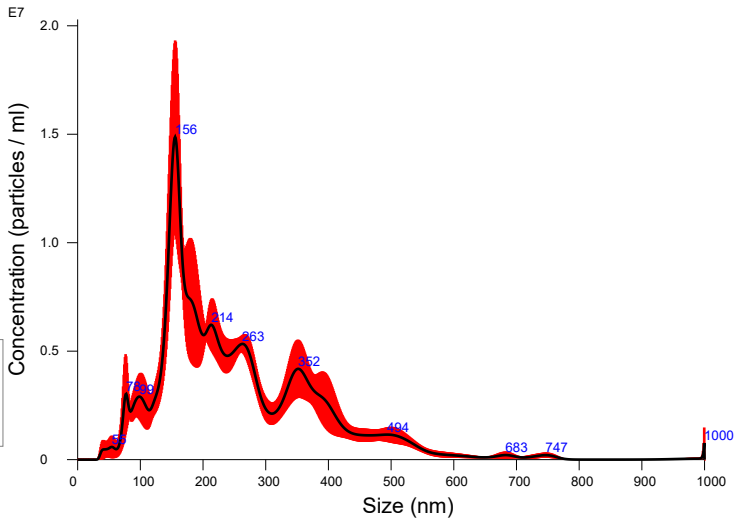
SOP Standard Measurement 01-40-07PM 14Apr2021.txt

NANOSIGHT

2021.04.27 MV ECA 10PE 2021-04-27 13-07-52



FTLA Concentration / Size graph for Experiment:
2021.04.27 MV ECA 10PE 2021-04-27 13-07-52



Averaged FTLA Concentration / Size for Experiment:
2021.04.27 MV ECA 10PE 2021-04-27 13-07-52
Error bars indicate + / -1 standard error of the mean

Included Files

2021.04.27 MV ECA 10PE 2021-04-27 13-10-29
2021.04.27 MV ECA 10PE 2021-04-27 13-11-38
2021.04.27 MV ECA 10PE 2021-04-27 13-12-47
2021.04.27 MV ECA 10PE 2021-04-27 13-13-56
2021.04.27 MV ECA 10PE 2021-04-27 13-15-06

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 01-07-49PM 27A~
Time Captured:	13:07:52 27/04/2021
Operator:	Hanna
Pre-treatment:	1:10
Sample Name:	MV ECA 10PE
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	11
Slider Shutter:	890
Slider Gain:	146
FPS	25.0
Number of Frames:	1498
Temperature:	25.1 - 25.2 ºC
Viscosity:	(Water) 0.885 - 0.888 cP
Dilution factor:	1 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 11.6 - 15.2 pix

Results

Stats: Merged Data

Mean:	254.4 nm
Mode:	155.4 nm
SD:	129.0 nm
D10:	131.2 nm
D50:	220.7 nm
D90:	422.3 nm

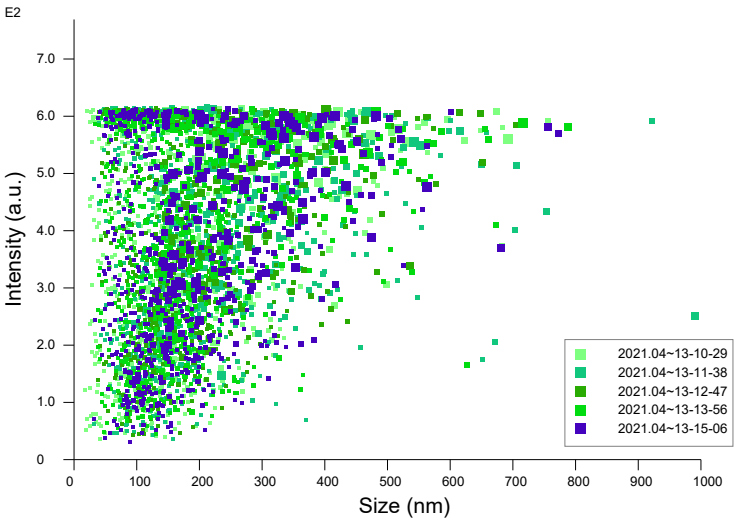
Stats: Mean +/- Standard Error

Mean:	254.4 +/- 5.0 nm
Mode:	163.8 +/- 7.1 nm
SD:	127.6 +/- 5.6 nm
D10:	131.3 +/- 6.3 nm
D50:	220.1 +/- 7.4 nm
D90:	433.0 +/- 11.3 nm
Concentration (Upgrade):	1.80e+09 +/- 5.89e+07 particles/ml
	19.7 +/- 0.7 particles/frame
	29.5 +/- 1.8 centres/frame

Concentration measurements may be unreliable
See summary file for more info

NANOSIGHT

2021.04.27 MV ECA 10PE 2021-04-27 13-07-52



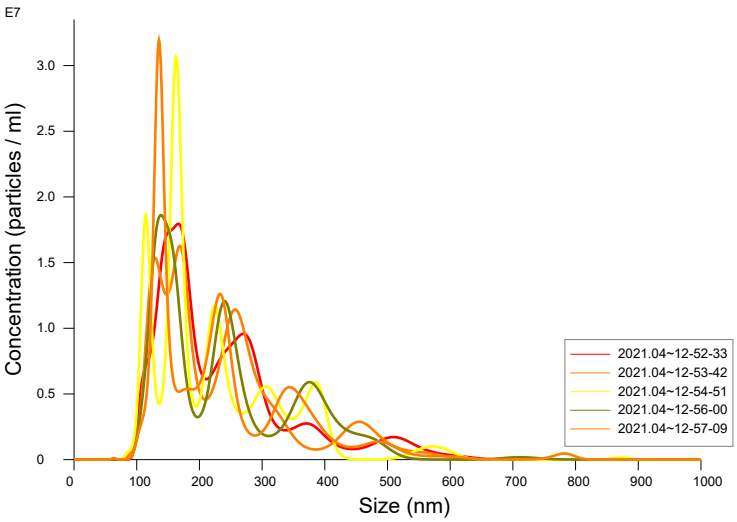
Intensity / Size graph for Experiment:
2021.04.27 MV ECA 10PE 2021-04-27 13-07-52

Script Used: (Full Text):

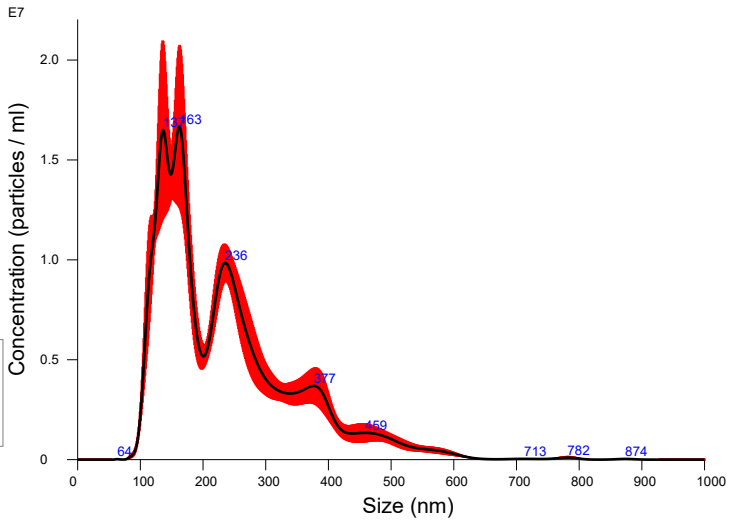
SOP Standard Measurement 01-07-49PM 27Apr2021.txt

NANOSIGHT

2021.04.27 ECA 10PE 2021-04-27 12-49-18



FTLA Concentration / Size graph for Experiment:
2021.04.27 ECA 10PE 2021-04-27 12-49-18



Averaged FTLA Concentration / Size for Experiment:
2021.04.27 ECA 10PE 2021-04-27 12-49-18
Error bars indicate + / -1 standard error of the mean

Included Files

2021.04.27 ECA 10PE 2021-04-27 12-52-33
2021.04.27 ECA 10PE 2021-04-27 12-53-42
2021.04.27 ECA 10PE 2021-04-27 12-54-51
2021.04.27 ECA 10PE 2021-04-27 12-56-00
2021.04.27 ECA 10PE 2021-04-27 12-57-09

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 12-48-58PM 27A~
Time Captured:	12:49:18 27/04/2021
Operator:	Hanna
Pre-treatment:	1:10
Sample Name:	MV ECV 6PE
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	12
Slider Shutter:	1200
Slider Gain:	146
FPS	25.0
Number of Frames:	1498
Temperature:	24.9 - 25.0 ºC
Viscosity:	(Water) 0.888 - 0.891 cP
Dilution factor:	1 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 9.6 - 11.0 pix

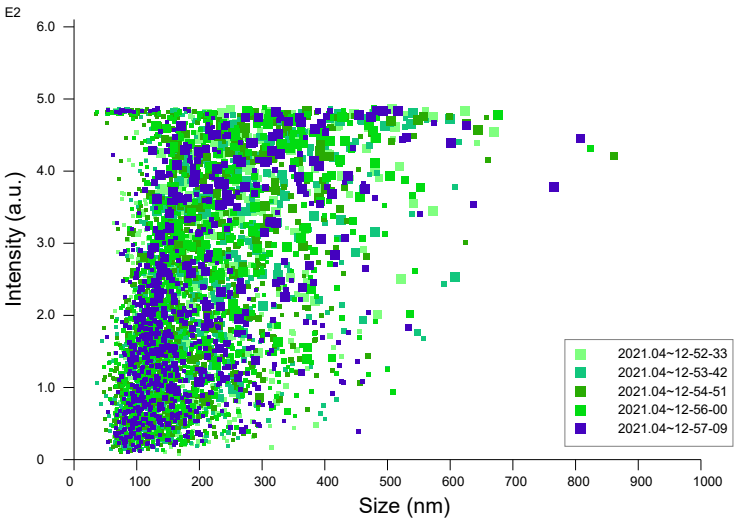
Results

Stats: Merged Data

Mean:	236.3 nm
Mode:	162.2 nm
SD:	110.9 nm
D10:	127.5 nm
D50:	210.1 nm
D90:	391.3 nm

Stats: Mean +/- Standard Error

Mean:	236.4 +/- 2.2 nm
Mode:	154.4 +/- 7.2 nm
SD:	110.9 +/- 2.1 nm
D10:	127.2 +/- 2.6 nm
D50:	209.2 +/- 5.2 nm
D90:	396.0 +/- 7.4 nm
Concentration (Upgrade):	2.42e+09 +/- 4.54e+07 particles/ml
	23.3 +/- 0.5 particles/frame
	24.5 +/- 0.6 centres/frame



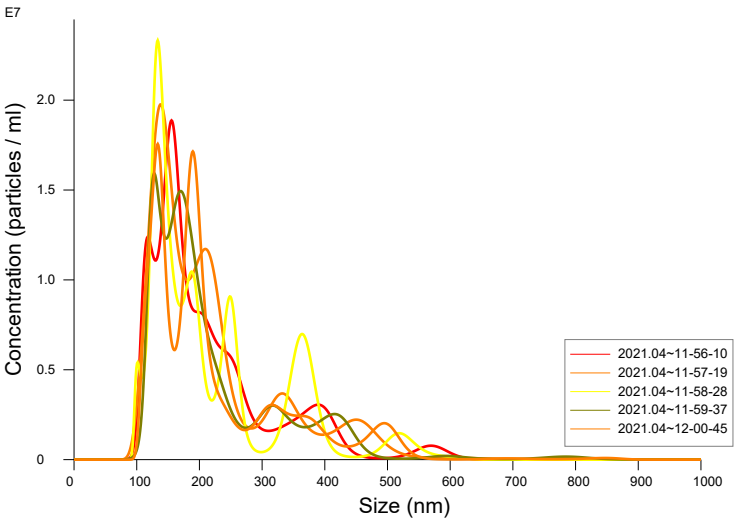
Intensity / Size graph for Experiment:
2021.04.27 ECA 10PE 2021-04-27 12-49-18

Script Used: (Full Text):

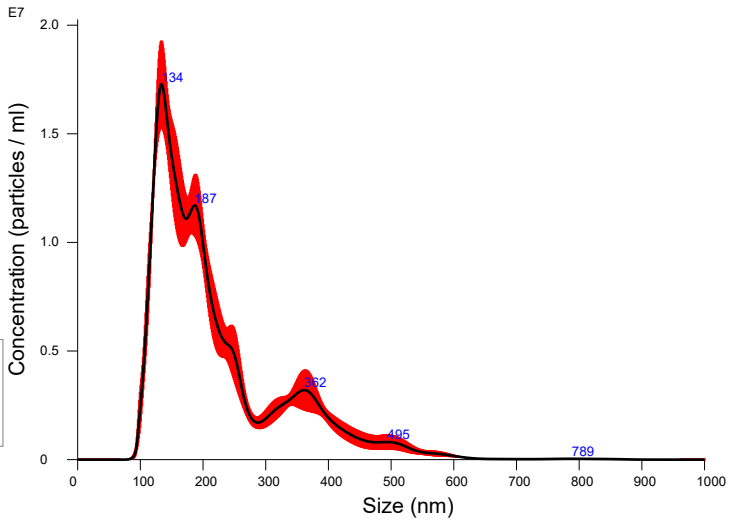
SOP Standard Measurement 12-48-58PM 27Apr2021.txt

NANOSIGHT

2021.04.27 MV ECV 10-2PE 2021-04-27 11-53-18



FTLA Concentration / Size graph for Experiment:
2021.04.27 MV ECV 10-2PE 2021-04-27 11-53-18



Averaged FTLA Concentration / Size for Experiment:
2021.04.27 MV ECV 10-2PE 2021-04-27 11-53-18
Error bars indicate + / -1 standard error of the mean

Included Files

2021.04.27 MV ECV 10-2PE 2021-04-27 11-56-10
2021.04.27 MV ECV 10-2PE 2021-04-27 11-57-19
2021.04.27 MV ECV 10-2PE 2021-04-27 11-58-28
2021.04.27 MV ECV 10-2PE 2021-04-27 11-59-37
2021.04.27 MV ECV 10-2PE 2021-04-27 12-00-45

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 11-52-53AM 27A~
Time Captured:	11:53:18 27/04/2021
Operator:	Hanna
Pre-treatment:	1:5
Sample Name:	MV ECV 10-2PE
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	11
Slider Shutter:	890
Slider Gain:	146
FPS	25.0
Number of Frames:	1498
Temperature:	24.8 - 25.0 ºC
Viscosity:	(Water) 0.888 - 0.893 cP
Dilution factor:	5 x 10e0
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 10.8 - 11.3 pix

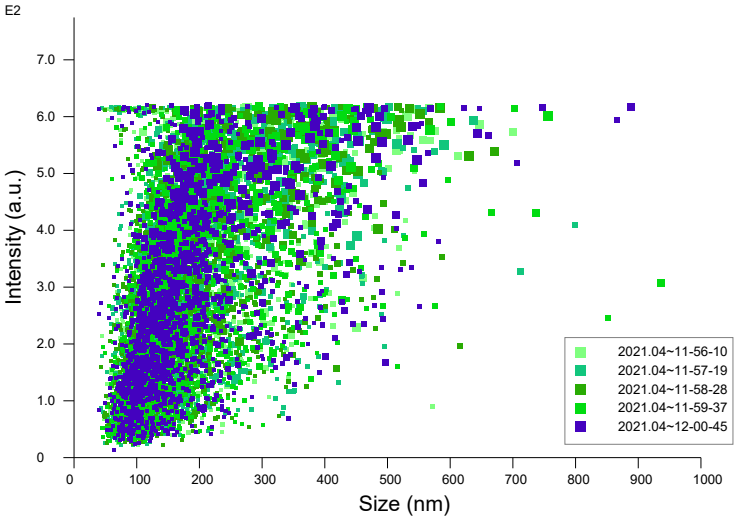
Results

Stats: Merged Data

Mean:	220.9 nm
Mode:	133.7 nm
SD:	107.6 nm
D10:	124.5 nm
D50:	185.5 nm
D90:	380.4 nm

Stats: Mean +/- Standard Error

Mean:	221.1 +/- 2.1 nm
Mode:	137.7 +/- 4.7 nm
SD:	107.7 +/- 2.7 nm
D10:	124.4 +/- 0.8 nm
D50:	185.0 +/- 1.6 nm
D90:	382.4 +/- 4.0 nm
Concentration (Upgrade):	2.08e+09 +/- 7.07e+07 particles/ml
	39.5 +/- 1.5 particles/frame
	40.9 +/- 1.5 centres/frame



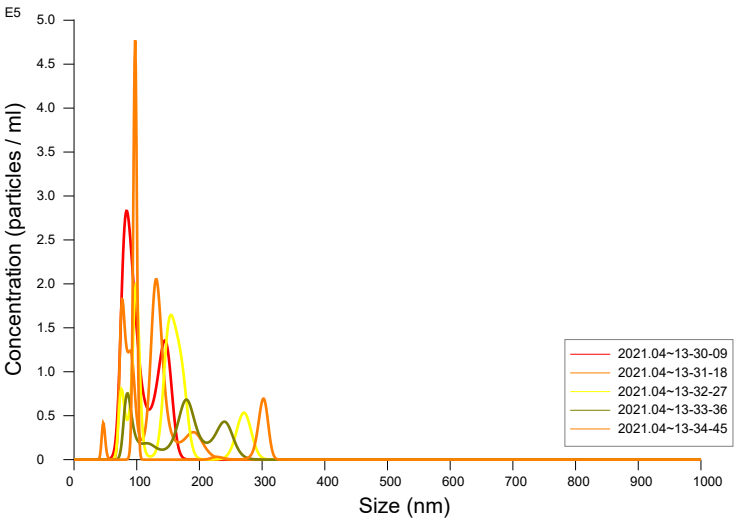
Intensity / Size graph for Experiment:
2021.04.27 MV ECV 10-2PE 2021-04-27 11-53-18

Script Used: (Full Text):

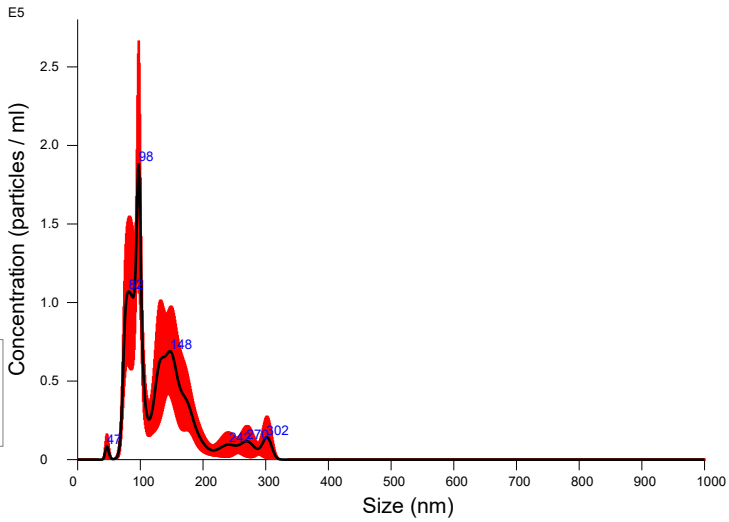
SOP Standard Measurement 11-52-53AM 27Apr2021.txt

NANOSIGHT

2021.04.27 PBS 2021-04-27 13-27-25



FTLA Concentration / Size graph for Experiment:
2021.04.27 PBS 2021-04-27 13-27-25



Averaged FTLA Concentration / Size for Experiment:
2021.04.27 PBS 2021-04-27 13-27-25
Error bars indicate + / -1 standard error of the mean

Included Files

2021.04.27 PBS 2021-04-27 13-30-09
2021.04.27 PBS 2021-04-27 13-31-18
2021.04.27 PBS 2021-04-27 13-32-27
2021.04.27 PBS 2021-04-27 13-33-36
2021.04.27 PBS 2021-04-27 13-34-45

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 01-26-56PM 27A~
Time Captured:	13:27:25 27/04/2021
Operator:	Hanna
Pre-treatment:	
Sample Name:	PBS
Diluent:	
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	25.3 - 25.5 ºC
Viscosity:	(Water) 0.879 - 0.883 cP
Dilution factor:	Dilution not recorded
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 11.0 - 14.2 pix

Results

Stats: Merged Data

Mean:	134.9 nm
Mode:	97.2 nm
SD:	57.0 nm
D10:	79.9 nm
D50:	123.1 nm
D90:	218.6 nm

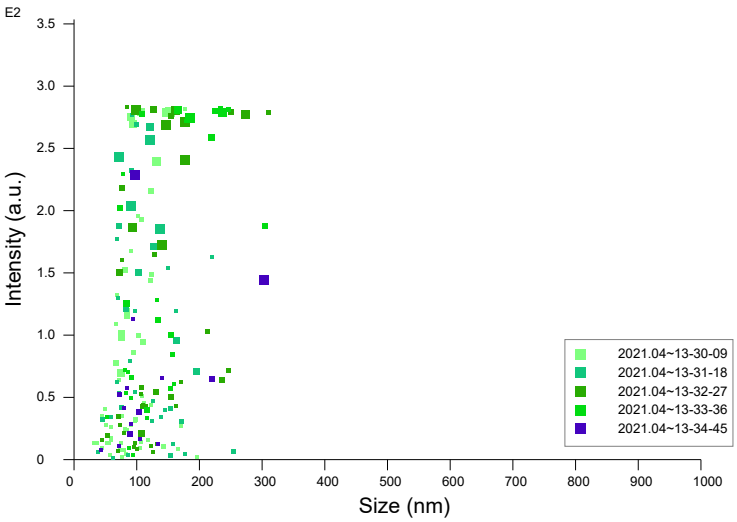
Stats: Mean +/- Standard Error

Mean:	140.7 +/- 11.7 nm
Mode:	99.3 +/- 8.5 nm
SD:	54.2 +/- 10.9 nm
D10:	84.3 +/- 3.4 nm
D50:	130.0 +/- 15.4 nm
D90:	226.8 +/- 28.7 nm
Concentration (Upgrade):	9.08e+06 +/- 1.49e+06 particles/ml
	0.9 +/- 0.2 particles/frame
	1.0 +/- 0.2 centres/frame

Concentration measurements may be unreliable
See summary file for more info

NANOSIGHT

2021.04.27 PBS 2021-04-27 13-27-25



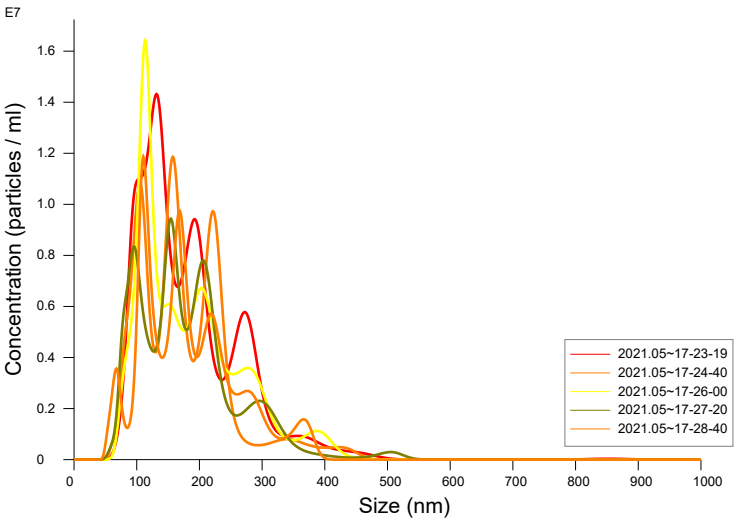
Intensity / Size graph for Experiment:
2021.04.27 PBS 2021-04-27 13-27-25

Script Used: (Full Text):

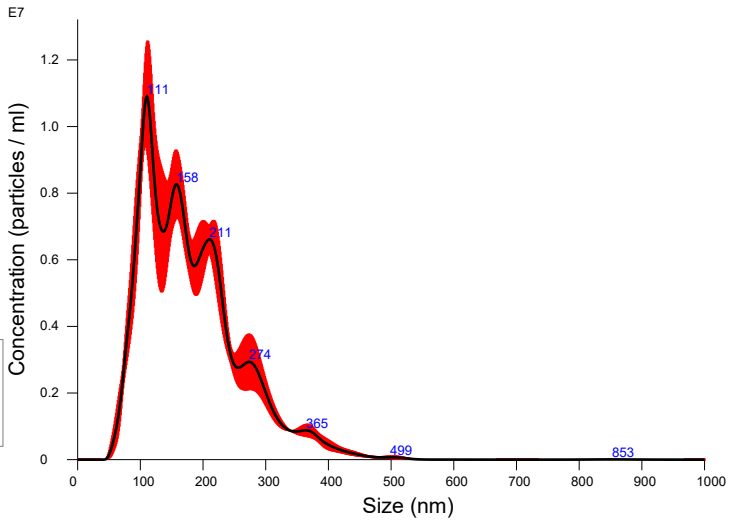
SOP Standard Measurement 01-26-56PM 27Apr2021.txt

NANOSIGHT

2021.05.06 MV ECA 16PE 2021-05-06 17-20-30



FTLA Concentration / Size graph for Experiment:
2021.05.06 MV ECA 16PE 2021-05-06 17-20-30



Averaged FTLA Concentration / Size for Experiment:
2021.05.06 MV ECA 16PE 2021-05-06 17-20-30
Error bars indicate + / -1 standard error of the mean

Included Files

2021.05.06 MV ECA 16PE 2021-05-06 17-23-19
2021.05.06 MV ECA 16PE 2021-05-06 17-24-40
2021.05.06 MV ECA 16PE 2021-05-06 17-26-00
2021.05.06 MV ECA 16PE 2021-05-06 17-27-20
2021.05.06 MV ECA 16PE 2021-05-06 17-28-40

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 05-19-58PM 06~
Time Captured:	17:20:30 06/05/2021
Operator:	Waltraud
Pre-treatment:	1:5
Sample Name:	MV ECA 16PE
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	23.0 - 23.3 ºC
Viscosity:	(Water) 0.924 - 0.930 cP
Dilution factor:	5 x 10e0
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 11.1 - 13.4 pix

Results

Stats: Merged Data

Mean:	181.4 nm
Mode:	110.4 nm
SD:	78.8 nm
D10:	97.1 nm
D50:	166.3 nm
D90:	287.9 nm

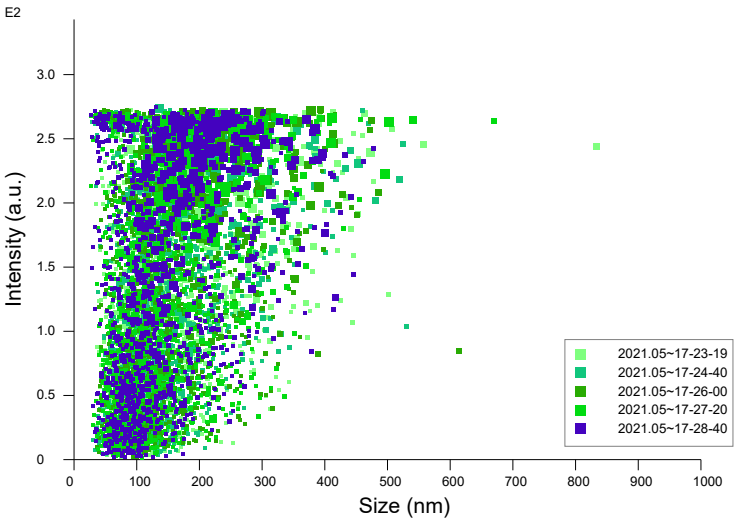
Stats: Mean +/- Standard Error

Mean:	181.3 +/- 1.5 nm
Mode:	133.5 +/- 9.9 nm
SD:	78.4 +/- 1.6 nm
D10:	97.3 +/- 2.1 nm
D50:	166.2 +/- 1.4 nm
D90:	283.1 +/- 8.6 nm
Concentration (Upgrade):	1.45e+09 +/- 9.90e+07 particles/ml
	31.2 +/- 2.1 particles/frame
	38.8 +/- 2.9 centres/frame

Concentration measurements may require some caution due to noise
See summary file for more info

NANOSIGHT

2021.05.06 MV ECA 16PE 2021-05-06 17-20-30



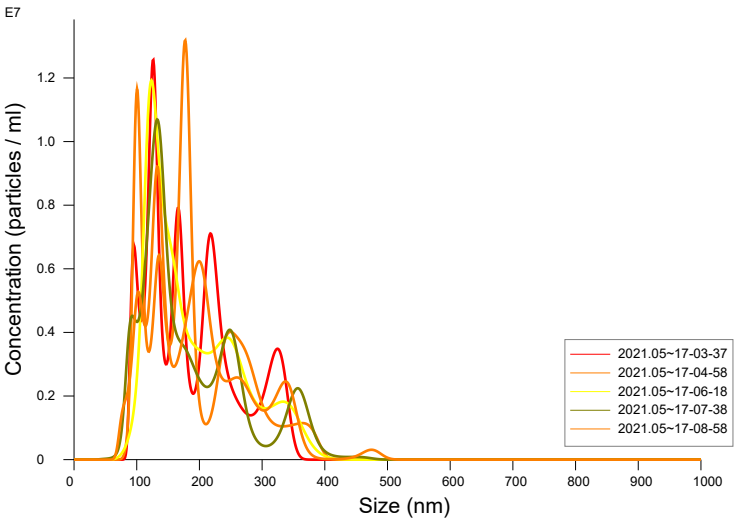
Intensity / Size graph for Experiment:
2021.05.06 MV ECA 16PE 2021-05-06 17-20-30

Script Used: (Full Text):

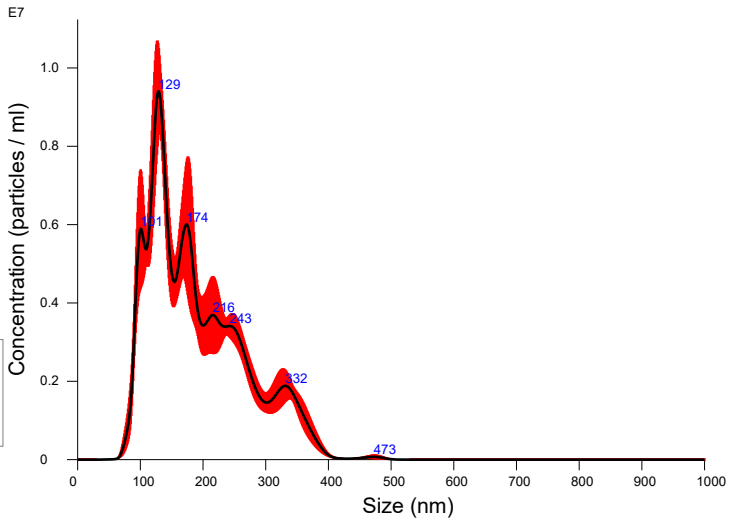
SOP Standard Measurement 05-19-58PM 06May2021.txt

NANOSIGHT

2021.05.06 MV ECV 14PE 2021-05-06 17-00-44



FTLA Concentration / Size graph for Experiment:
2021.05.06 MV ECV 14PE 2021-05-06 17-00-44



Averaged FTLA Concentration / Size for Experiment:
2021.05.06 MV ECV 14PE 2021-05-06 17-00-44
Error bars indicate + / -1 standard error of the mean

Included Files

2021.05.06 MV ECV 14PE 2021-05-06 17-03-37
2021.05.06 MV ECV 14PE 2021-05-06 17-04-58
2021.05.06 MV ECV 14PE 2021-05-06 17-06-18
2021.05.06 MV ECV 14PE 2021-05-06 17-07-38
2021.05.06 MV ECV 14PE 2021-05-06 17-08-58

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 04-59-54PM 06~
Time Captured:	17:00:44 06/05/2021
Operator:	Waltraud
Pre-treatment:	1:5
Sample Name:	MV ECV 14PE
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	23.0 - 23.3 ºC
Viscosity:	(Water) 0.925 - 0.930 cP
Dilution factor:	5 x 10e0
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 10.7 - 11.8 pix

Results

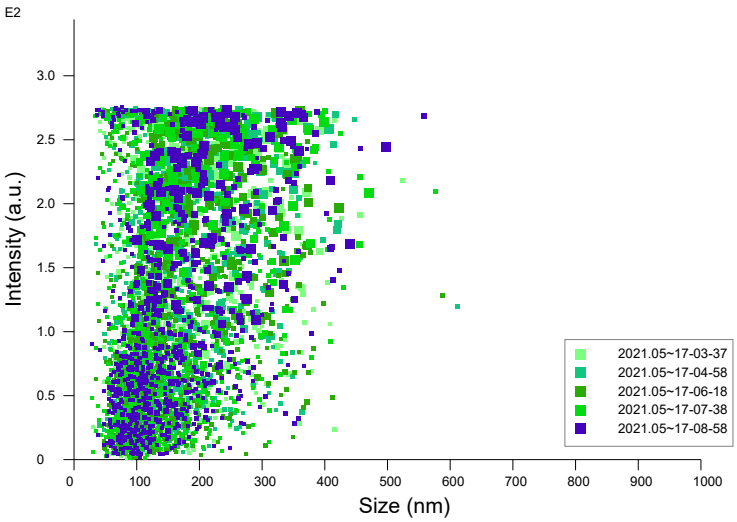
Stats: Merged Data

Mean:	190.9 nm
Mode:	129.0 nm
SD:	76.6 nm
D10:	106.4 nm
D50:	173.2 nm
D90:	311.2 nm

Stats: Mean +/- Standard Error

Mean:	190.9 +/- 1.7 nm
Mode:	138.5 +/- 9.8 nm
SD:	76.6 +/- 2.1 nm
D10:	106.6 +/- 2.4 nm
D50:	171.9 +/- 5.0 nm
D90:	313.6 +/- 6.3 nm
Concentration (Upgrade):	1.07e+09 +/- 2.02e+07 particles/ml
	22.0 +/- 0.4 particles/frame
	26.3 +/- 0.6 centres/frame

Concentration measurements may require some caution due to noise
See summary file for more info



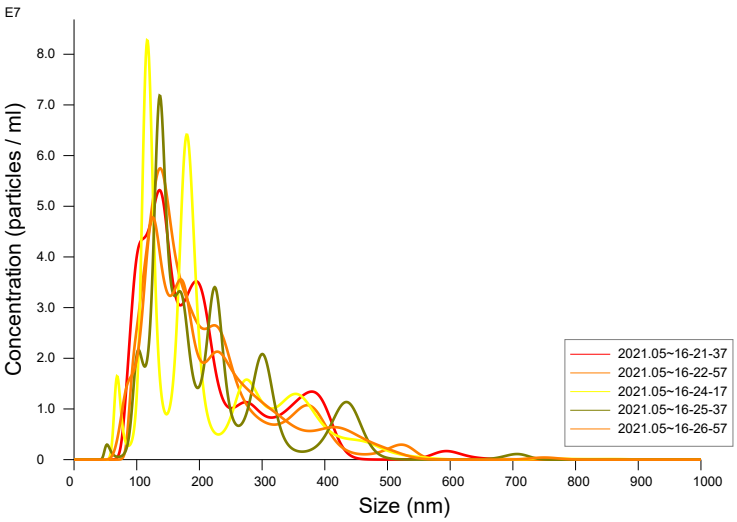
Intensity / Size graph for Experiment:
2021.05.06 MV ECV 14PE 2021-05-06 17-00-44

Script Used: (Full Text):

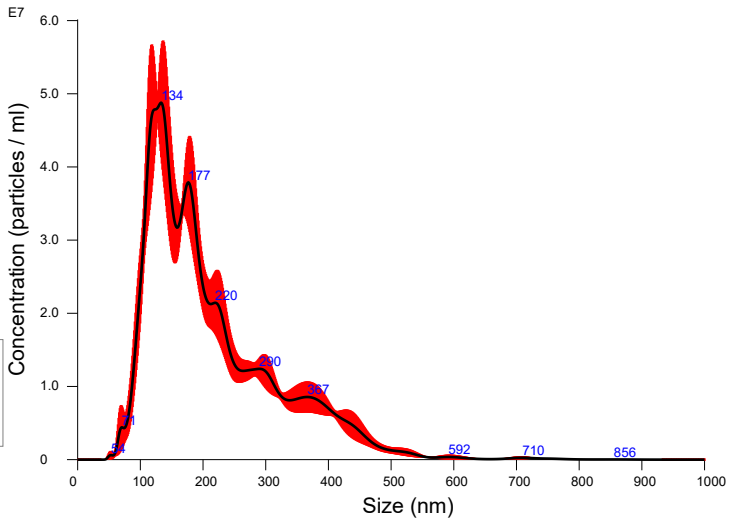
SOP Standard Measurement 04-59-54PM 06May2021.txt

NANOSIGHT

2021.05.06 MV ECV 17PE 2021-05-06 16-18-48



FTLA Concentration / Size graph for Experiment:
2021.05.06 MV ECV 17PE 2021-05-06 16-18-48



Averaged FTLA Concentration / Size for Experiment:
2021.05.06 MV ECV 17PE 2021-05-06 16-18-48
Error bars indicate + / -1 standard error of the mean

Included Files

2021.05.06 MV ECV 17PE 2021-05-06 16-21-37
2021.05.06 MV ECV 17PE 2021-05-06 16-22-57
2021.05.06 MV ECV 17PE 2021-05-06 16-24-17
2021.05.06 MV ECV 17PE 2021-05-06 16-25-37
2021.05.06 MV ECV 17PE 2021-05-06 16-26-57

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 04-18-41PM 06~
Time Captured:	16:18:48 06/05/2021
Operator:	Waltraud
Pre-treatment:	1:10
Sample Name:	MV ECV 17PE
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	12
Slider Shutter:	1200
Slider Gain:	146
FPS	25.0
Number of Frames:	1498
Temperature:	22.7 - 22.9 ºC
Viscosity:	(Water) 0.933 - 0.938 cP
Dilution factor:	1 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 10.5 - 12.3 pix

Results

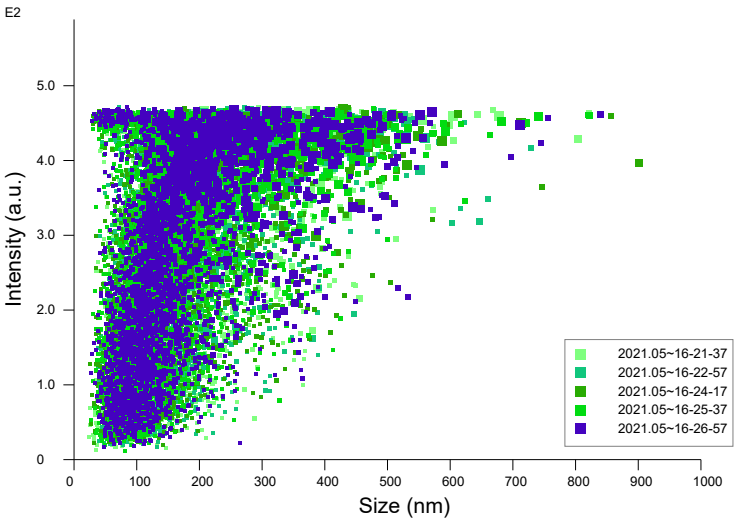
Stats: Merged Data

Mean:	212.3 nm
Mode:	133.1 nm
SD:	105.0 nm
D10:	111.2 nm
D50:	180.6 nm
D90:	371.6 nm

Stats: Mean +/- Standard Error

Mean:	212.6 +/- 2.7 nm
Mode:	130.7 +/- 4.1 nm
SD:	105.0 +/- 2.5 nm
D10:	112.4 +/- 2.7 nm
D50:	180.4 +/- 1.6 nm
D90:	377.1 +/- 7.6 nm
Concentration (Upgrade):	7.03e+09 +/- 1.81e+08 particles/ml
	77.2 +/- 1.3 particles/frame
	103.3 +/- 1.4 centres/frame

Concentration measurements may require some caution due to noise
See summary file for more info



Intensity / Size graph for Experiment:
2021.05.06 MV ECV 17PE 2021-05-06 16-18-48

Script Used: (Full Text):

SOP Standard Measurement 04-18-41PM 06May2021.txt

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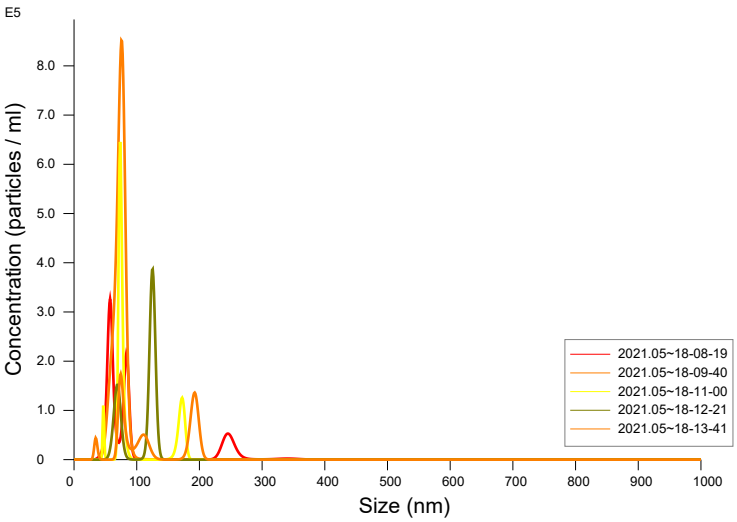
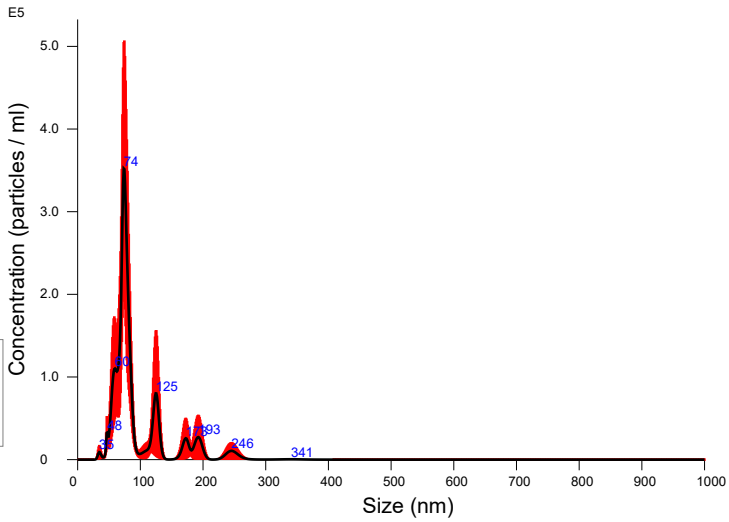
2021.05.06 PBS 2021-05-06 18-05-42



FTLA Concentration / Size graph for Experiment:
2021.05.06 PBS 2021-05-06 18-05-42



Averaged FTLA Concentration / Size for Experiment:
2021.05.06 PBS 2021-05-06 18-05-42
Error bars indicate + / -1 standard error of the mean

Included Files

2021.05.06 PBS 2021-05-06 18-08-19
2021.05.06 PBS 2021-05-06 18-09-40
2021.05.06 PBS 2021-05-06 18-11-00
2021.05.06 PBS 2021-05-06 18-12-21
2021.05.06 PBS 2021-05-06 18-13-41

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 05-59-02PM 06~
Time Captured:	18:05:42 06/05/2021
Operator:	Waltraud
Pre-treatment:	
Sample Name:	PBS
Diluent:	PBS
Remarks:	

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	15
Slider Shutter:	1206
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	23.3 - 23.5 ºC
Viscosity:	(Water) 0.920 - 0.925 cP
Dilution factor:	Dilution not recorded
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 14.2 pix

Results

Stats: Merged Data

Mean:	94.2 nm
Mode:	74.0 nm
SD:	46.6 nm
D10:	58.7 nm
D50:	76.5 nm
D90:	173.0 nm

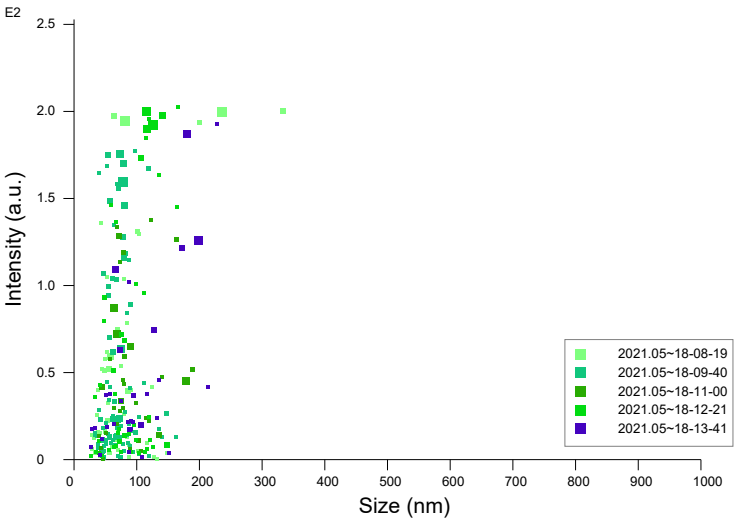
Stats: Mean +/- Standard Error

Mean:	100.6 +/- 8.5 nm
Mode:	81.3 +/- 11.5 nm
SD:	41.5 +/- 11.2 nm
D10:	64.1 +/- 3.3 nm
D50:	90.1 +/- 10.5 nm
D90:	165.6 +/- 27.9 nm
Concentration (Upgrade):	8.74e+06 +/- 1.87e+06 particles/ml
	0.8 +/- 0.2 particles/frame
	0.8 +/- 0.2 centres/frame

Concentration measurements may be unreliable
See summary file for more info

NANOSIGHT

2021.05.06 PBS 2021-05-06 18-05-42



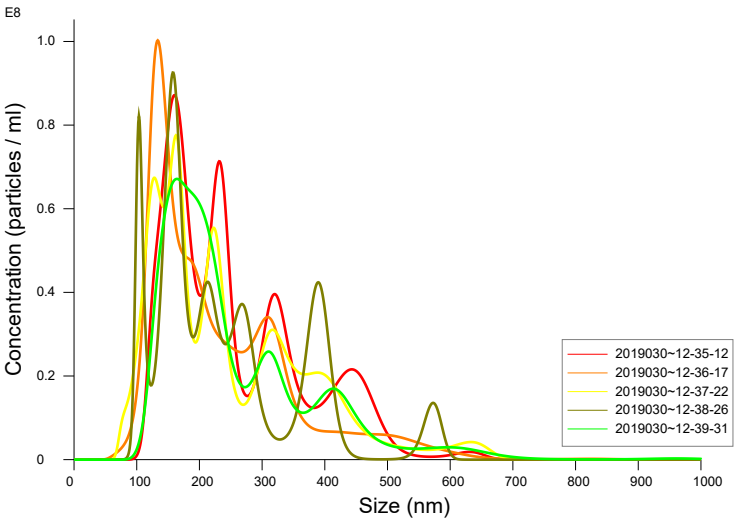
Intensity / Size graph for Experiment:
2021.05.06 PBS 2021-05-06 18-05-42

Script Used: (Full Text):

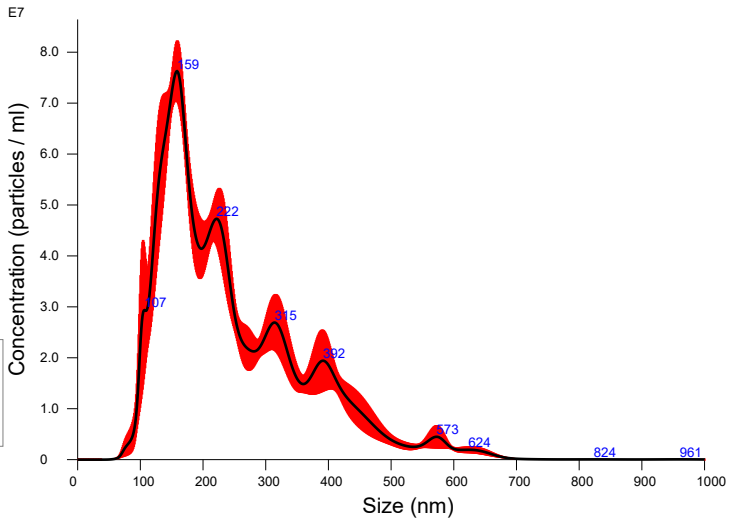
SOP Standard Measurement 05-59-02PM 06May2021.txt

NANOSIGHT

20190301_MV_ECV20 1-100 2019-03-01 12-32-31



FTLA Concentration / Size graph for Experiment:
20190301_MV_ECV20 1-100 2019-03-01 12-32-31



Averaged FTLA Concentration / Size for Experiment:
20190301_MV_ECV20 1-100 2019-03-01 12-32-31
Error bars indicate + / -1 standard error of the mean

Included Files

20190301_MV_ECV20 1-100 2019-03-01 12-35-12
20190301_MV_ECV20 1-100 2019-03-01 12-36-17
20190301_MV_ECV20 1-100 2019-03-01 12-37-22
20190301_MV_ECV20 1-100 2019-03-01 12-38-26
20190301_MV_ECV20 1-100 2019-03-01 12-39-31

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 11-08-36AM 01~
Time Captured:	12:32:31 01/03/2019
Operator:	
Pre-treatment:	1:100 dilution
Sample Name:	100 nm blue laser Flow cell
Diluent:	PBS
Remarks:	ECV20 MV

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	12
Slider Shutter:	1200
Slider Gain:	146
FPS	25.0
Number of Frames:	1498
Temperature:	24.9 - 25.1 ºC
Viscosity:	(Water) 0.886 - 0.890 cP
Dilution factor:	5 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 9.3 - 11.9 pix

Results

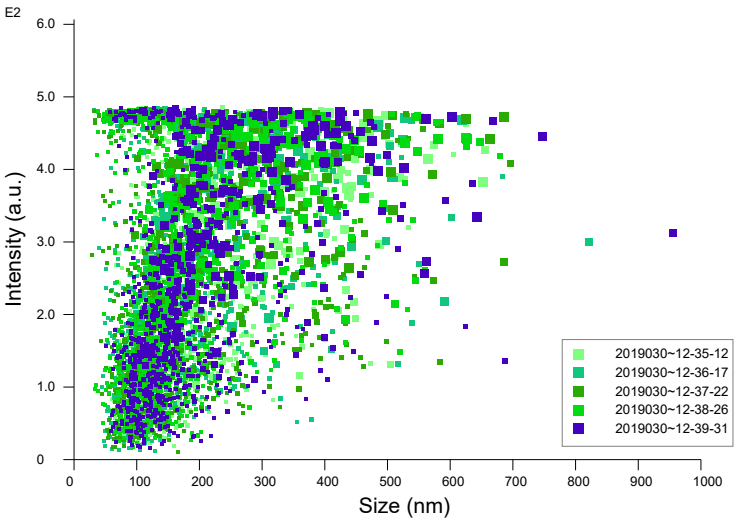
Stats: Merged Data

Mean:	246.8 nm
Mode:	158.4 nm
SD:	118.3 nm
D10:	128.7 nm
D50:	214.0 nm
D90:	414.0 nm

Stats: Mean +/- Standard Error

Mean:	246.7 +/- 4.8 nm
Mode:	155.7 +/- 5.6 nm
SD:	117.9 +/- 2.6 nm
D10:	128.1 +/- 6.1 nm
D50:	212.5 +/- 5.2 nm
D90:	412.2 +/- 8.8 nm
Concentration (Upgrade):	1.22e+10 +/- 4.04e+08 particles/ml
	25.9 +/- 0.8 particles/frame
	30.6 +/- 0.8 centres/frame

Concentration measurements may require some caution due to noise
See summary file for more info



Intensity / Size graph for Experiment:
20190301_MV_ECV20 1-100 2019-03-01 12-32-31

Script Used: (Full Text):

SOP Standard Measurement 11-08-36AM 01Mar2019.txt

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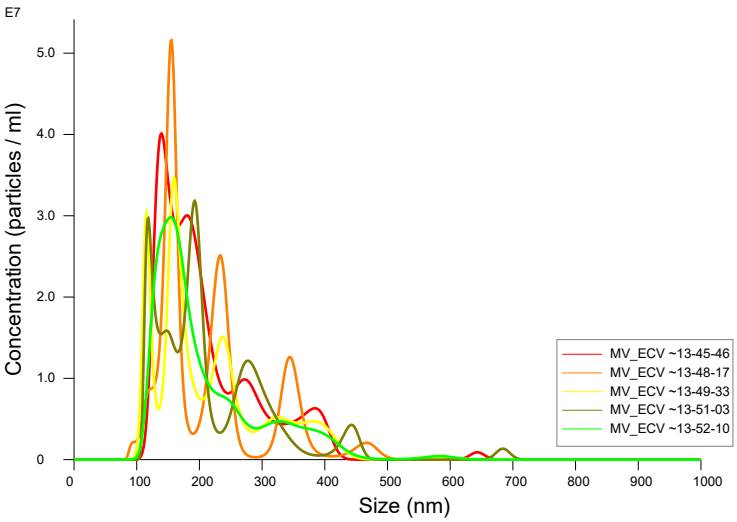
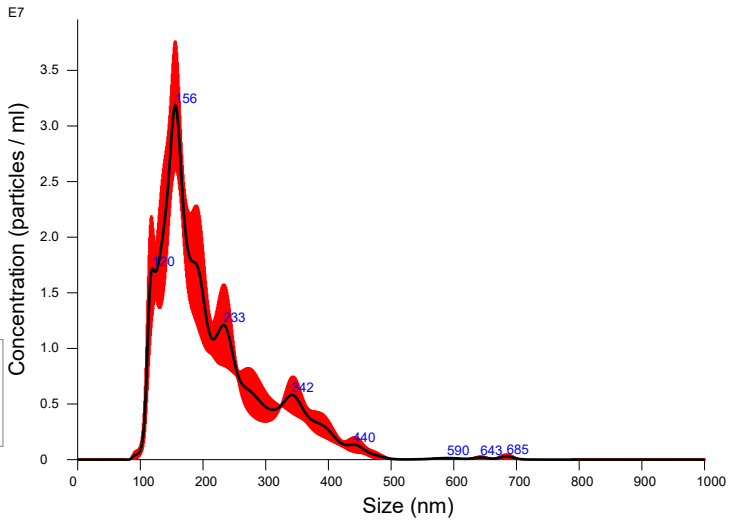
MV_ECV 84_1 10 2019-02-22 13-42-56



FTLA Concentration / Size graph for Experiment:
MV_ECV 84_1 10 2019-02-22 13-42-56



Averaged FTLA Concentration / Size for Experiment:
MV_ECV 84_1 10 2019-02-22 13-42-56
Error bars indicate + / -1 standard error of the mean

Included Files

MV_ECV 84_1 10 2019-02-22 13-45-46
MV_ECV 84_1 10 2019-02-22 13-48-17
MV_ECV 84_1 10 2019-02-22 13-49-33
MV_ECV 84_1 10 2019-02-22 13-51-03
MV_ECV 84_1 10 2019-02-22 13-52-10

Details

NTA Version:	NTA 3.3 Dev Build 3.3.301
Script Used:	SOP Standard Measurement 01-39-23PM 22~
Time Captured:	13:42:56 22/02/2019
Operator:	Vi
Pre-treatment:	1:10
Sample Name:	Blue Laser
Diluent:	PBS
Remarks:	ECV 84

Capture Settings

Camera Type:	sCMOS
Laser Type:	Blue488
Camera Level:	11
Slider Shutter:	890
Slider Gain:	146
FPS	25.0
Number of Frames:	1498
Temperature:	22.0 ºC
Viscosity:	(Water) 1.0 cP
Dilution factor:	1 x 10e1
Syringe Pump Speed:	50

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 9.9 - 10.9 pix

Results

Stats: Merged Data

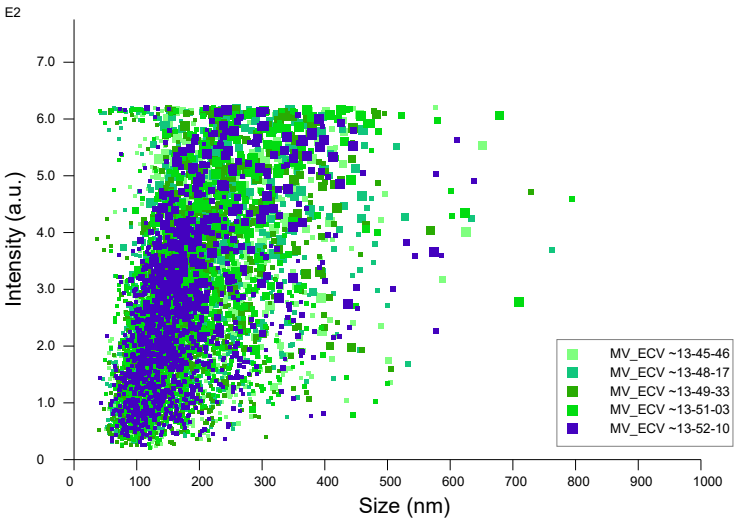
Mean:	214.3 nm
Mode:	155.8 nm
SD:	88.3 nm
D10:	129.2 nm
D50:	186.4 nm
D90:	346.2 nm

Stats: Mean +/- Standard Error

Mean:	214.5 +/- 1.5 nm
Mode:	160.1 +/- 8.7 nm
SD:	88.4 +/- 2.2 nm
D10:	128.8 +/- 3.7 nm
D50:	186.1 +/- 2.6 nm
D90:	344.2 +/- 5.4 nm
Concentration (Upgrade):	3.37e+09 +/- 2.06e+08 particles/ml
	30.7 +/- 1.6 particles/frame
	31.9 +/- 1.6 centres/frame

NANOSIGHT

MV_ECV 84_1 10 2019-02-22 13-42-56



Intensity / Size graph for Experiment:
MV_ECV 84_1 10 2019-02-22 13-42-56

Script Used: (Full Text):

SOP Standard Measurement 01-39-23PM 22Feb2019.txt

5. BCA measurements

The total protein concentration per sample was determined with a Pierce BCA™ Protein Assay Kit (Thermo Fisher) and with the help of an absorbance microplate reader. A standard curve was determined by bovine serum albumin (BSA) standard samples. Technical triplicates were utilized. As reference, seven standard samples, with given protein concentrations, were added to every measurement (125, 250, 500, 750, 1000, 1500, and 2000 µg/ml). The experiment was performed according to the manufacturer's protocol. 5 µl sample and 200 µl prepared working reagent were used per well. Solutions were mixed on a plate shaker for 30 sec and then incubated at 37 °C for 30 minutes. Before measurement, the plate was cooled down to room temperature. The plate reader measured the absorbance at 562 nm. The average result was calculated as µg/ml. The accuracy has been proved by using the standard curve.

Table 4: 1st set of samples.

Well	Content	Blank corrected based on Raw Data (562)	Average over replicates based on Blank corrected (562)	Linear regression fit based on Blank corrected in µg/ml (562)
A01	Blank B			
A02	Blank B			
A03	Blank B			
A04	Medium	0,192	0,185	1022,26
A05	Medium	0,192	0,185	1021,929
A06	Medium	0,17	0,185	949,403
A07	ECV212	0,348	0,338	1538,555
A08	ECV212	0,337	0,338	1500,801
A09	ECV212	0,33	0,338	1479,275
A10	ECV150	-0,013	-0,012	342,699
A11	ECV150	-0,018	-0,012	327,134
A12	ECV150	-0,004	-0,012	372,504
B04	ECV42	0,061	0,062	588,427
B05	ECV42	0,054	0,062	564,252
B06	ECV42	0,07	0,062	617,239
B07	ECV158	-0,048	-0,053	226,458
B08	ECV158	-0,047	-0,053	230,101
B09	ECV158	-0,065	-0,053	172,478
B10	ECV159	0,027	0,031	474,836
B11	ECV159	0,035	0,031	501,33
B12	ECV159	0,033	0,031	494,706
C04	ECA212	-0,007	-0,005	363,894
C05	ECA212	-0,009	-0,005	356,608
C06	ECA212	0,002	-0,005	392,043
C07	ECA150	-0,069	-0,063	156,913
C08	ECA150	-0,064	-0,063	174,465
C09	ECA150	-0,057	-0,063	196,984
C10	ECA42	-0,039	-0,034	256,264
C11	ECA42	-0,031	-0,034	284,082
C12	ECA42	-0,032	-0,034	281,433
D04	ECA158	-0,014	-0,003	338,725
D05	ECA158	0,006	-0,003	406,284
D06	ECA158	-0,002	-0,003	380,453
D07	ECA159	0,012	0,017	424,167
D08	ECA159	0,017	0,017	443,044
D09	ECA159	0,023	0,017	460,927
D10	ECA18PE	-0,0005	0,0007	384,427
D11	ECA18PE	0,003	0,0007	395,686
D12	ECA18PE	-0,0003	0,0007	385,089
E04	ECA1PE	0,029	0,033	480,797
E05	ECA1PE	0,043	0,033	529,479
E06	ECA1PE	0,027	0,033	476,161
E07	ECA10PE	0,069	0,08	615,583
E08	ECA10PE	0,086	0,08	672,213
E09	ECA10PE	0,084	0,08	665,921
H01	2000	0,474	0,48	1955,167
H02	2000	0,47	0,48	1943,576
H03	2000	0,497	0,48	2030,342
G01	1500	0,327	0,328	1469,009
G02	1500	0,327	0,328	1470,002
G03	1500	0,33	0,328	1478,944
F01	1000	0,184	0,187	997,091
F02	1000	0,209	0,187	1076,903
F03	1000	0,167	0,187	939,468
E01	750	0,151	0,147	885,156
E02	750	0,153	0,147	894,429
E03	750	0,136	0,147	835,812
D01	500	0,043	0,04	528,154
D02	500	0,039	0,04	513,583
D03	500	0,039	0,04	516,563
C01	250	-0,052	-0,054	213,874
C02	250	-0,056	-0,054	201,289
C03	250	-0,055	-0,054	204,932
B01	125	-0,1	-0,094	53,919
B02	125	-0,095	-0,094	72,464
B03	125	-0,088	-0,094	94,321

Table 5: Standard curve of 1st set of samples.

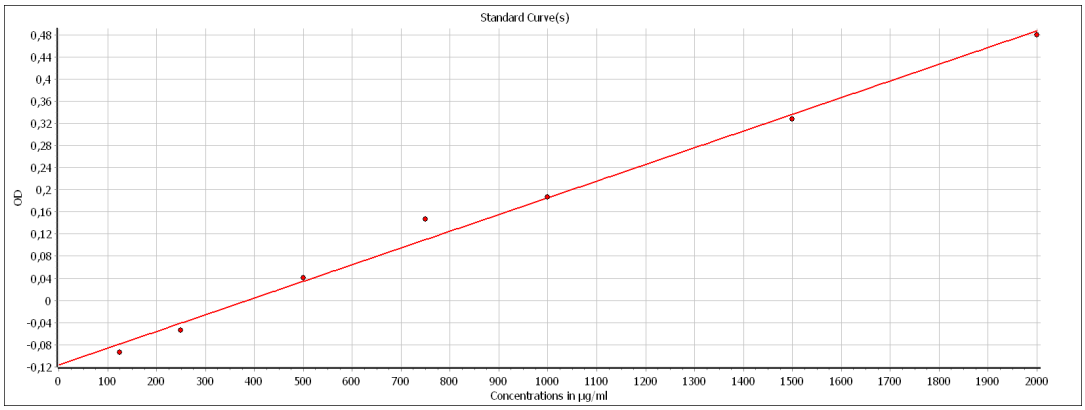


Table 6: 2nd set of samples.

Well	Content	Blank corrected based on Raw Data (562)	Average over replicates based on Blank corrected (562)	L+A11:E54ar regression fit based on Blank corrected in µg/ml (562)
A01	Blank B			
A02	Blank B			
A03	Blank B			
A04	ECV 42	0,018	0,012	98,468
A05	ECV 42	0,006	0,012	60,172
A06	ECV 14 PE	0,039	0,038	164,527
A07	ECV 14 PE	0,037	0,038	158,783
A08	ECA 10 PE	0,389	0,357	1280,522
A09	ECA 10 PE	0,341	0,357	1129,574
A10	ECA 10 PE	0,341	0,357	1129,574
B04	ECV 10-2 PE	0,125	0,167	440,574
B05	ECV 10-2 PE	0,199	0,167	676,73
B06	ECV 10-2 PE	0,175	0,167	597,905
B07	ECV 6 PE	0,092	0,087	335,262
B08	ECV 6 PE	0,093	0,087	337,176
B09	ECV 6 PE	0,076	0,087	281,648
B10	ECV 17 PE	0,153	0,174	529,93
B11	ECV 17 PE	0,161	0,174	552,588
B12	ECV 17 PE	0,208	0,174	705,451
C04	MV ECV 159	0,134	0,149	466,424
C05	MV ECV 159	0,177	0,149	604,287
C06	MV ECV 159	0,137	0,149	476,317
H01	2000	0,591	0,592	1925,801
H02	2000	0,529	0,592	1727,622
H03	2000	0,656	0,592	2134,511
G01	1500	0,525	0,507	1716,133
G02	1500	0,513	0,507	1678,476
G03	1500	0,482	0,507	1577,631
F01	1000	0,223	0,278	753,002
F02	1000	0,284	0,278	947,032
F03	1000	0,327	0,278	1084,896
E01	750	0,209	0,21	707,047
E02	750	0,207	0,21	700,983
E03	750	0,215	0,21	727,152
D01	500	0,148	0,146	512,697
D02	500	0,142	0,146	494,188
D03	500	0,148	0,146	512,059
C01	250	0,063	0,067	240,161
C02	250	0,073	0,067	272,712
C03	250	0,065	0,067	246,224
B01	125	0,031	0,031	138,359
B02	125	0,033	0,031	145,699
B03	125	0,029	0,031	132,615

Table 7: Standard curve of 2nd set of samples.

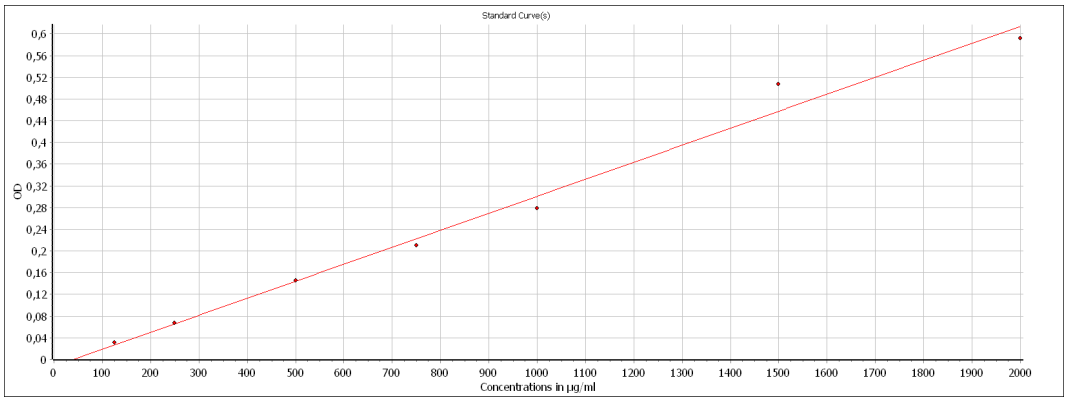
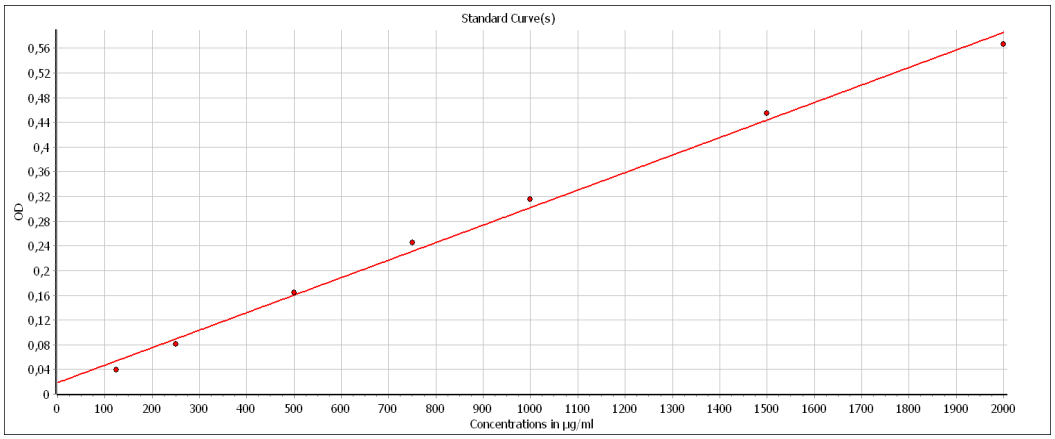


Table 8: 3rd set of samples

Well	Content	Blank corrected based on Raw Data (562)	Average over replicates based on Blank corrected (562)	Linear regression fit based on Blank corrected in µg/ml (562)
A01	Blank B			
A02	Blank B			
A03	Blank B			
A04	ECV 212	0,488	0,497	1658,036
A05	ECV 212	0,481	0,497	1630,867
A06	ECV 212	0,52	0,497	1770,597
A07	Medium	0,037	0,045	63,483
A08	Medium	0,041	0,045	79,009
A09	Medium	0,058	0,045	140,758
H01	2000	0,577	0,566	1970,666
H02	2000	0,558	0,566	1903,624
H03	2000	0,563	0,566	1921,619
G01	1500	0,41	0,455	1379,634
G02	1500	0,443	0,455	1497,487
G03	1500	0,512	0,455	1739,546
F01	1000	0,315	0,315	1047,244
F02	1000	0,318	0,315	1055,007
F03	1000	0,313	0,315	1038,07
E01	750	0,26	0,245	850,351
E02	750	0,227	0,245	736,731
E03	750	0,249	0,245	814,006
D01	500	0,137	0,164	417,75
D02	500	0,183	0,164	579,005
D03	500	0,174	0,164	547,953
C01	250	0,095	0,081	270,609
C02	250	0,076	0,081	201,45
C03	250	0,071	0,081	184,16
B01	125	0,039	0,039	70,54
B02	125	0,044	0,039	91,359
B03	125	0,035	0,039	58,19

Table 9: Standard curve of 3rd set of samples.



Samples ECV 20, 56, 84 as well as ECA 217 and 208 have been measured by another lab employee in advance. The measured protein concentrations (average of triplicates/duplicates) in µg/ml are listed in the following table.

Sample Liste	µg/ml
ECA 212	0,37
ECA 150	0,18
ECA 158	0,38
ECA 159	0,44
ECA 42	0,27
ECV 212	1,51
ECV 150	0,35
ECV 158	0,21
ECV 159	0,49
ECV 42	0,59
ECA 1 PE	0,50
ECA 10 PE	0,65
ECA 18 PE	0,39
Medium	1,00
ECA 16 PE	0,08
ECA 10 PE (2)	1,18
ECV 10-2 PE	0,57
ECV 6 PE	0,32
ECV 14 PE	0,16
ECV 17 PE	0,60
MV ECV 159	0,52
ECV 20	0,55
ECV 56	0,38
ECV 84	0,56
ECA 217	0,66
ECA 208	1,61
ECV 212	1,69

ECA 16 PE has been excluded since there was not enough protein for the western blot measurements.

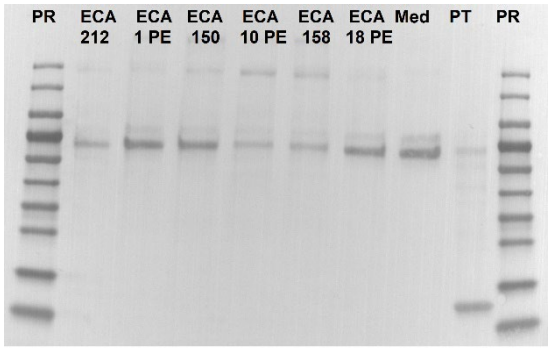
Samples were measured in 4 different western blot experiments:

Nr.	WB 1	WB 2	WB 3	WB 4
1	ECA 212	ECV 212	ECA 159	ECV 20
2	ECA 1 PE	ECV 6 PE	ECA 42	ECA217
3	ECA 150	ECV 150	ECA 10 PE (2)	ECV 56
4	ECA 10 PE	ECV 14 PE	ECV 212	ECA 208
5	ECA 158	ECV 158	ECV 42	ECV 84
6	ECA 18 PE	ECV 17 PE	ECV 10-2 PE	ECV 212
7	Medium	ECV 159	MV ECV 159	Medium

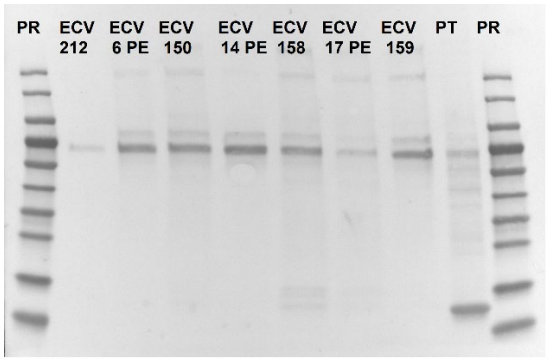
6. Ponceau staining:

Abbreviations: WB: western blot; EAC: arterial endothelial cells, ECV: venous endothelial cells; The added numbers were given from our lab in order of isolation of the primary endothelial cells; PE: pregnancy diagnosed with preeclampsia; Med: medium; MV: microvesicles; PT: placental tissue; PR: page ruler

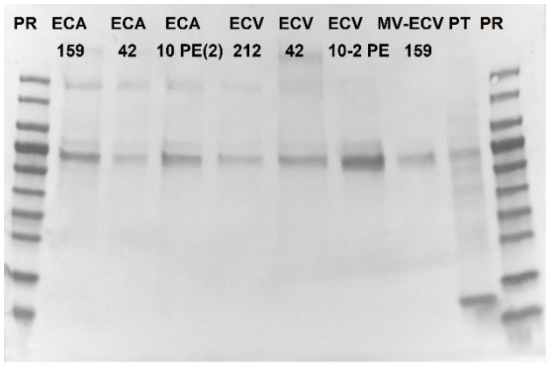
WB 1



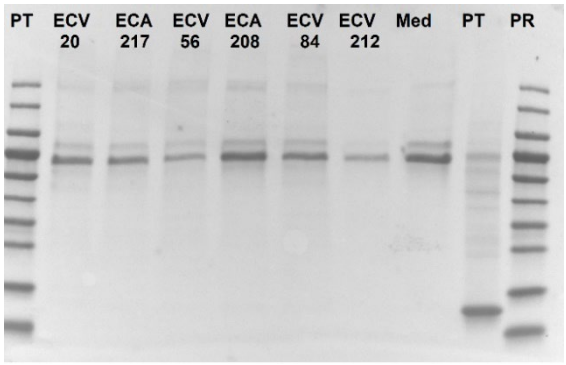
WB 2



WB 3



WB 4



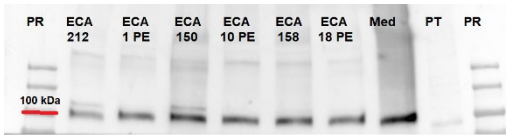
7. Western Blot (WB)

Western blot experiments were performed according to the standard operating procedure of our lab (chapter 10)

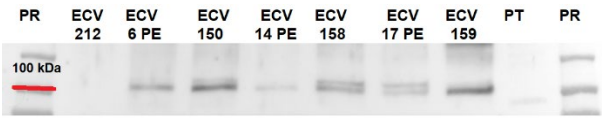
Abbreviations: EAC: arterial endothelial cells, ECV: venous endothelial cells; The added numbers were given from our lab in order of isolation of the primary endothelial cells; PE: pregnancy diagnosed with preeclampsia; Med: medium; MV: microvesicles; PT: placental tissue; PR: page ruler

ALIX

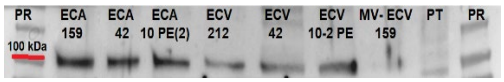
WB 1



WB 2



WB 3

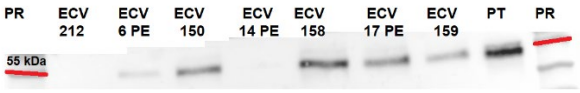


WB 4

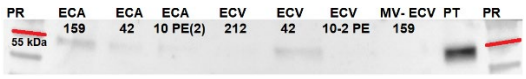
TSG101

WB 1

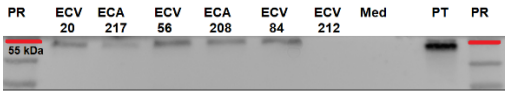
WB 2



WB 3

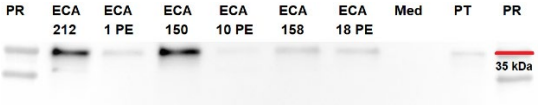


WB 4

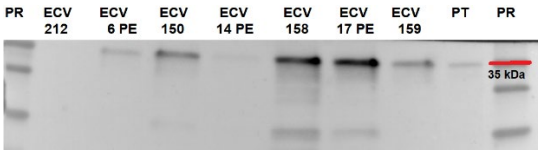


Syntenin-1

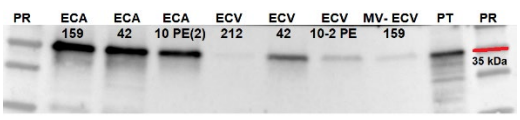
WB 1



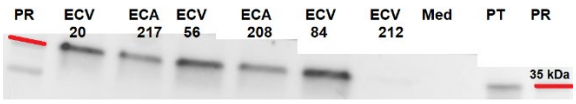
WB 2



WB 3

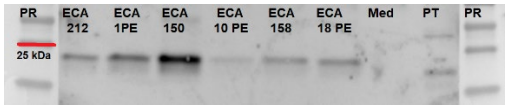


WB 4

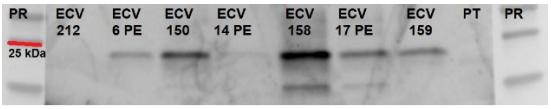


CD81

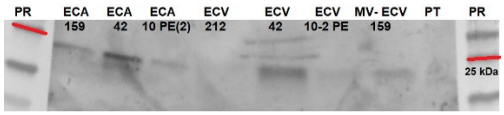
WB 1



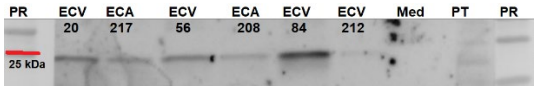
WB 2



WB 3



WB 4



Negative controls:

PLAP

WB 1

WB 2

WB 3

WB 4

GRP94

WB 1

WB 2

WB 3

WB 4

The protein bands of all proteins showed up in the expected weight areas.

ECV 212 did not reveal the investigated protein bands as expected, so we performed the western blot experiment three times. But, as shown in the following protein measurements, ECV 212 showed way less protein compared to the other samples each time. Consequently, we excluded this sample from the statistics.

In the first Nanosight tracking analysis the ECA 10 PE sample showed impurity. We decided to perform all measurements for this sample a second time, which revealed better results. We used the repeated measurements (ECA 10 PE (2)) for statistical analysis.

8. Referring the volume of the protein bands to the total protein volume from Ponceau staining

Abbreviations: WB: western blot; Vol: volume; P: Ponceau; Ref: volume of investigated protein referred to the total protein volume; EAC: arterial endothelial cells, ECV: venous endothelial cells; The added numbers were given from our lab in order of isolation of the primary endothelial cells; PE: pregnancy diagnosed with preeclampsia; Med: medium; MV: microvesicles; PT: placental tissue

WB 1 + 2

WB1
ALIX/Ponceau

	AlixVol	Pvol	Ref
ECA 212	9.631.323	485.200.274	0,0198502
ECA 1PE	15.890.025	465.136.395	0,03416208
ECA 150	22.243.251	466.877.450	0,04764259
ECA 10PE	17.721.372	515.143.455	0,03440085
ECA158	18.805.555	508.311.572	0,03699612
ECA 18PE	17.390.850	448.499.427	0,03877563
Medium	27.879.093	444.217.517	0,06276
PT	2.027.294	377.184.817	0,0053748

TSG101/Ponceau

	TSGVol	Pvol	Ref
ECA 212	3.830.261	485.200.274	0,00789419
ECA 1PE	41.839.952	465.136.395	0,08995201
ECA 150	16.412.788	466.877.450	0,03515438
ECA 10PE	55.692.036	515.143.455	0,10810976
ECA158	10.452.154	508.311.572	0,02056249
ECA 18PE	17.074.165	448.499.427	0,03806954
Medium	9.229.441	444.217.517	0,02077685

syntenin-1/Ponceau

	SyntVol	Pvol	Ref
ECA 212	18.392.953	327.194.072	0,0562142
ECA 1PE	2.119.158	414.604.572	0,00511128
ECA 150	24.219.717	386.153.750	0,0627204
ECA 10PE	906.603	415.540.876	0,00218174
ECA158	2.976.212	411.089.108	0,00723982
ECA 18PE	2.327.483	395.418.145	0,00588613
Medium	259.359	360.332.641	0,00071978
PT	1.034.603	329.379.064	0,00314107

CD81/Ponceau

	CD81Vol	Pvol	Ref
ECA 212	13.851.021	494.161.587	0,02802934
ECA 1PE	23.501.976	538.821.013	0,04361741
ECA 150	58.040.964	548.940.993	0,10573261
ECA 10PE	4.104.566	579.801.719	0,00707926
ECA158	9.972.720	576.205.883	0,01730756
ECA 18PE	10.903.801	551.671.568	0,01976502

WB2
ALIX/Ponceau

	AlixVol	Pvol	Ref
ECV 212	2.318.248	509.281.407	0,004552
ECV 6 PE	26.355.831	516.180.360	0,05105934
ECV 150	57.477.628	489.466.759	0,11742907
ECV 14 PE	8.133.866	517.478.432	0,01571827
ECV 158	42.509.796	525.235.295	0,08093477
ECV 17 PE	23.278.205	585.186.782	0,0397791
ECV 159	57.571.704	520.857.284	0,11053259
PT	2.631.562	516.224.507	0,00509771

TSG101/Ponceau

	TSGVol	Pvol	Ref
ECV 212	400.210	509.281.407	0,00078583
ECV 6 PE	5.953.283	516.180.360	0,01153334
ECV 150	36.516.700	489.466.759	0,07460507
ECV 14 PE	1.471.520	517.478.432	0,00284364
ECV 158	64.709.180	525.235.295	0,12320036
ECV 17 PE	39.130.838	585.186.782	0,06686897
ECV 159	22.575.904	520.857.284	0,04334374

syntenin-1/Ponceau

	SyntVol	Pvol	Ref
ECV 212	210.198	465.720.777	0,00045134
ECV 6 PE	1.706.212	483.994.658	0,00352527
ECV 150	6.780.260	429.707.709	0,01577877
ECV 14 PE	759.952	464.848.349	0,00163484
ECV 158	18.511.735	457.017.276	0,04050555
ECV 17 PE	20.974.649	497.791.544	0,04213541
ECV 159	5.196.762	436.338.529	0,01190993
PT	1.109.977	399.114.009	0,0027811

CD81/Ponceau

	CD81Vol	Pvol	Ref
ECV 212	3.642.845	393.889.706	0,00924839
ECV 6 PE	8.865.022	449.021.219	0,01974299
ECV 150	27.980.037	448.998.681	0,06231652
ECV 14 PE	5.181.233	447.856.593	0,01156896
ECV 158	58.042.014	430.704.706	0,13476058
ECV 17 PE	21.602.155	489.923.404	0,04409292
ECV 159	19.823.793	468.428.923	0,04231975

WB 3 + 4

WB3
ALIX/Ponceau

	AlixVol	Pvol	Ref
ECA 159	21.900.889	578.660.517	0,03784756
ECA 42	16.877.754	573.815.378	0,02941321
ECA 10 PE (2)	18.147.157	609.007.145	0,02979794
ECV 212	8.285.264	740.068.393	0,01119527
ECV 42	7.231.004	719.500.341	0,01005004
ECV 10-2 PE	7.414.290	557.844.580	0,01329096

TSG101/Ponceau

	TSGVol	Pvol	Ref
ECA 159	30.151.709	578.660.517	0,05210604
ECA 42	8.976.155	573.815.378	0,01564293
ECA 10 PE (2)	7.719.391	609.007.145	0,01267537
ECV 212	3.236.156	740.068.393	0,00437278
ECV 42	22.666.559	719.500.341	0,03150319
ECV 10-2 PE	3.106.822	557.844.580	0,00556933
MV ECV 159	1.613.892	620.465.269	0,0026011

syntenin-1/Ponceau

	SyntVol	Pvol	Ref
ECA 159	109.737.413	607.445.925	0,1806538
ECA 42	76.996.509	624.693.657	0,12325483
ECA 10 PE (2)	60.724.391	605.059.754	0,10036098
ECV 212	1.984.180	603.764.866	0,00328635
ECV 42	25.621.894	601.986.601	0,04256223
ECV 10-2 PE	6.155.253	556.761.273	0,01105546
MV ECV 159	3.292.866	628.577.440	0,0052386
PT	26.081.197	528.721.818	0,04932877

CD81/Ponceau

	CD81Vol	Pvol	Ref
ECA 159	8.367.946	448.676.862	0,01865027
ECA 42	10.670.298	496.953.588	0,02147142
ECA 10 PE (2)	4.332.646	481.739.081	0,00899376
ECV 212	1.878.957	594.326.353	0,00316149
ECV 42	9.213.956	586.334.266	0,01571451
ECV 10-2 PE	1.732.804	556.000.560	0,00311655
MV ECV 159	7.699.600	527.390.474	0,01459943

WB4
ALIX/Ponceau

	AlixVol	Pvol	Ref
ECV 20	13518801	291964068	0,04630296
ECA217	16129990	355991006	0,04531011
ECV56	20869254	344684614	0,06054594
ECA208	8765987	344058872	0,02547816
ECV84	26656694	343529572	0,0775965
ECV212	2052749	381721603	0,00537761
Medium	36417669	337092813	0,10803455

TSG101/Ponceau

	TSGVol	Pvol	Ref
ECV 20	26424576	291964068	0,09050626
ECA217	14070325	355991006	0,03952438
ECV56	30932076	344684614	0,08974023
ECA208	20798351	344058872	0,06044998
ECV84	29677864	343529572	0,086391
ECV212	808049	381721603	0,00211685
Medium	656316	337092813	0,00194699

syntenin-1/Ponceau

	SyntVol	Pvol	Ref
ECV 20	46839943	660856828	0,0708776
ECA217	22321947	706953680	0,03157484
ECV56	39935009	677976473	0,05890324
ECA208	22603240	771942546	0,02928099
ECV84	45623234	713336370	0,06395753
ECV212	1477869	667072797	0,00221545
Medium	829067	741423918	0,00111821
PT	11264562	586158082	0,01921762

CD81/Ponceau

	CD81Vol	Pvol	Ref
ECV 20	19224638	290830892	0,06610246
ECA217	10891123	348855584	0,03121957
ECV56	15161599	347702084	0,04360514
ECA208	8017712	328049213	0,02444058
ECV84	43883376	353435343	0,12416239
ECV212	4215449	375797320	0,01121735
Medium	1815423	347397029	0,00522579
PT	4857433	320865946	0,01513851

The referred results were used for statistical analysis.

9. Statistical analyses performed in SPSS (in German)

We first performed the descriptive statistical analysis.

Deskriptive Statistik

Deskriptive Statistik

	N	Minimum	Maximum	Mittelwert	Std.-Abweichung
PE	21	0	1	,33	,483
Vessel	21	0	1	,52	,512
Gültige Werte (Listenweise)	21				

Häufigkeiten

Statistiken

		PE	Vessel
N	Gültig	21	21
	Fehlend	0	0

Häufigkeitstabelle

PE

		Häufigkeit	Prozent	Gültige Prozente	Kumulierte Prozente
Gültig	nonPE	14	66,7	66,7	66,7
	PE	7	33,3	33,3	100,0
	Gesamt	21	100,0	100,0	

Vessel

		Häufigkeit	Prozent	Gültige Prozente	Kumulierte Prozente
Gültig	Arteriell	10	47,6	47,6	47,6
	Venös	11	52,4	52,4	100,0
	Gesamt	21	100,0	100,0	

Kreuztabellen

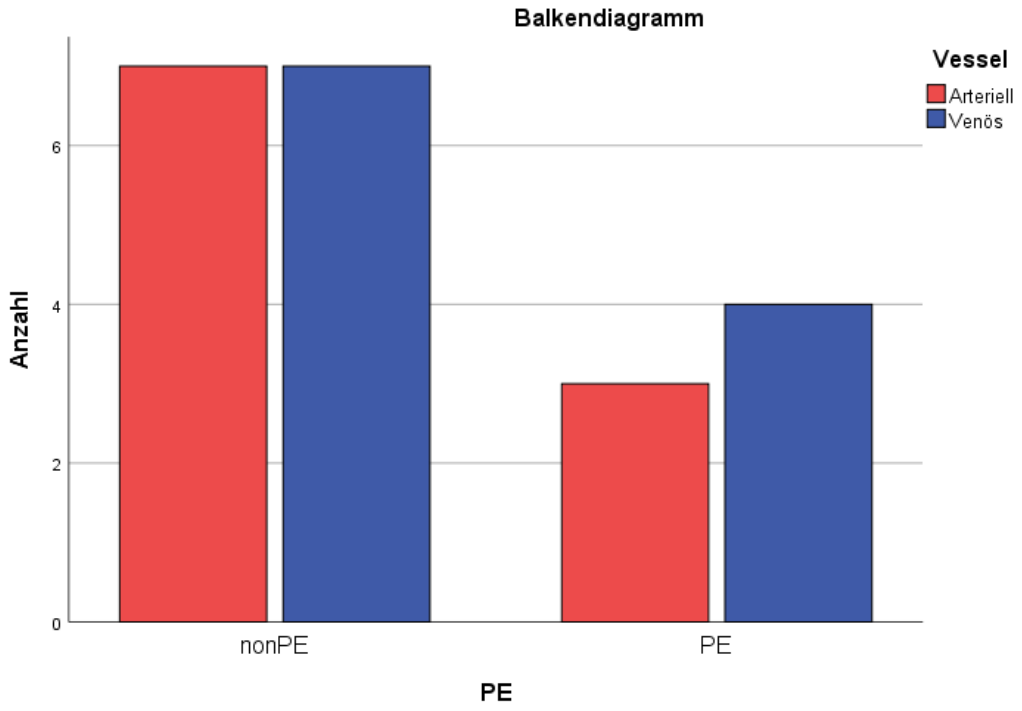
Verarbeitete Fälle

	Fälle					
	Gültig		Fehlend		Gesamt	
	N	Prozent	N	Prozent	N	Prozent
PE * Vessel	21	100,0%	0	0,0%	21	100,0%

PE * Vessel Kreuztabelle

Anzahl

		Vessel		
		Arteriell	Venös	Gesamt
PE	nonPE	7	7	14
	PE	3	4	7
Gesamt		10	11	21



In a second step, we performed inductive statistical analyses. With a total sample number of 21 and only seven in the smallest (PE) group, which represents a non-Gaussian distribution, groups were compared with the Man Whitney U Test, with a CI of 95%, and significance was considered $p < 0.05$.

Mann-Whitney- U Test

Analyses were performed for all proteins in both groups (pregnancy course and vessel type): ALIX, TSG 101, syntenin-1, CD81 referred to Ponceau (Pon) staining. Further, the comparisons of the size distribution (mode measured by the Nanosight instrument) and the total protein per exosom in fg in both groups.

Ränge

	Vessel	N	Mittlerer Rang	Rangsumme
PonAlix	Arteriell	10	8,80	88,00
	Venös	11	13,00	143,00
	Gesamt	21		
PonTSG	Arteriell	10	9,50	95,00
	Venös	11	12,36	136,00
	Gesamt	21		
PonSynt	Arteriell	10	12,10	121,00
	Venös	11	10,00	110,00
	Gesamt	21		
PonCD81	Arteriell	10	12,60	126,00
	Venös	11	9,55	105,00
	Gesamt	21		

Statistik für Test[a]

	PonAlix	PonTSG	PonSynt	PonCD81
Mann-Whitney-U	33,000	40,000	44,000	39,000
Wilcoxon-W	88,000	95,000	110,000	105,000
Z	-1,549	-1,056	-,775	-1,127
Asymptotische Signifikanz (2-seitig)	,121	,291	,439	,260
Exakte Signifikanz [2*(1-seitige Sig.)]	,132[b]	,314[b]	,468[b]	,282[b]

a. Gruppenvariable: Vessel

b. Nicht für Bindungen korrigiert.

Ränge

	PE	N	Mittlerer Rang	Rangsumme
PonAlix	nonPE	14	12,36	173,00
	PE	7	8,29	58,00
	Gesamt	21		
PonTSG	nonPE	14	12,50	175,00
	PE	7	8,00	56,00
	Gesamt	21		
PonSynt	nonPE	14	13,07	183,00
	PE	7	6,86	48,00
	Gesamt	21		
PonCD81	nonPE	14	11,86	166,00
	PE	7	9,29	65,00
	Gesamt	21		

Statistik für Test[a]

	PonAlix	PonTSG	PonSynt	PonCD81
Mann-Whitney-U	30,000	28,000	20,000	37,000
Wilcoxon-W	58,000	56,000	48,000	65,000
Z	-1,417	-1,567	-2,164	-,895
Asymptotische Signifikanz (2-seitig)	,156	,117	,030	,371
Exakte Signifikanz [2*(1-seitige Sig.)]	,172[b]	,128[b]	,031[b]	,400[b]

a. Gruppenvariable: PE

b. Nicht für Bindungen korrigiert.

Ränge

	PE	N	Mittlerer Rang	Rangsumme
Mode	nonPE	14	9,82	137,50
	PE	7	13,36	93,50
	Gesamt	21		

Statistik für Test[a]

	Mode
Mann-Whitney-U	32,500
Wilcoxon-W	137,500
Z	-1,232
Asymptotische Signifikanz (2-seitig)	,218
Exakte Signifikanz [2*(1-seitige Sig.)]	,224[b]

a. Gruppenvariable: PE

b. Nicht für Bindungen korrigiert.

Ränge

	Vessel	N	Mittlerer Rang	Rangsumme
Mode	Arteriell	10	8,55	85,50
	Venös	11	13,23	145,50
	Gesamt	21		

Statistik für Test[a]

	Mode
Mann-Whitney-U	30,500
Wilcoxon-W	85,500
Z	-1,726
Asymptotische Signifikanz (2-seitig)	,084
Exakte Signifikanz [2*(1-seitige Sig.)]	,085[b]

a. Gruppenvariable: Vessel

b. Nicht für Bindungen korrigiert.

Ränge

	PE	N	Mittlerer Rang	Rangsumme
ProtPerExfg	nonPE	14	9,50	133,00
	PE	7	14,00	98,00
	Gesamt	21		

Statistik für Test[a]

	ProtPerExfg
Mann-Whitney-U	28,000
Wilcoxon-W	133,000
Z	-1,567
Asymptotische Signifikanz (2-seitig)	,117
Exakte Signifikanz [2*(1-seitige Sig.)]	,128[b]

a. Gruppenvariable: PE

b. Nicht für Bindungen korrigiert.

Ränge

	Vessel	N	Mittlerer Rang	Rangsumme
ProtPerExfg	Arteriell	10	12,40	124,00
	Venös	11	9,73	107,00
	Gesamt	21		

Statistik für Test[a]

	ProtPerExfg
Mann-Whitney-U	41,000
Wilcoxon-W	107,000
Z	-,986
Asymptotische Signifikanz (2-seitig)	,324
Exakte Signifikanz [2*(1-seitige Sig.)]	,349[b]

a. Gruppenvariable: Vessel

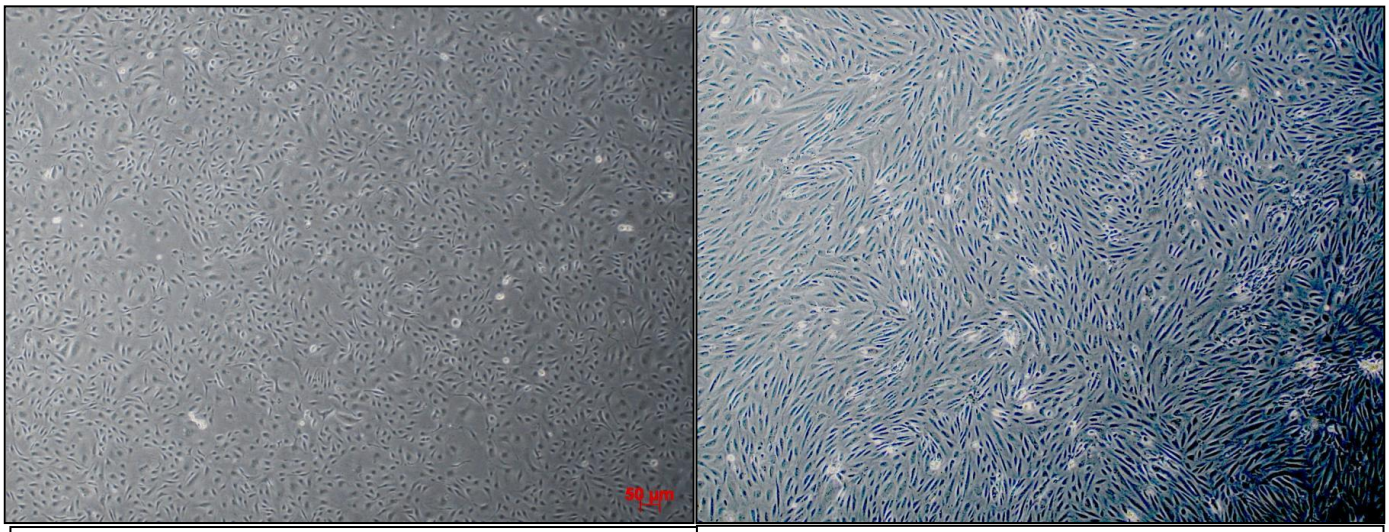
b. Nicht für Bindungen korrigiert.

10. Standard operating protocols from the PlacentaLab of LKH Graz

- Cultivation of human feto-placental endothelial cells (fpECs)
- Exosome Isolation from endothelial cell culture supernatants
- BCA Protein Analysis
- Nanosight NS3000
- Western blot Biorad

Medizinische Universität Graz

Cultivation of human feto-placental endothelial cells (fpECs)



ECA	ECV

Perinatal Research Laboratory

Department of Obstetrics & Gynecology

Medical University of Graz

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1. General cell culture rules

- Every person who starts working in a cell culture lab should be familiar with standard cell culture methods
- Don´t eat or drink in the cell culture lab
- Remove watch, rings and bracelets
- Always wear a labcoat and other personal protective equipment
- The door of the cell culture room should be closed while working
- Wash and disinfect your hands before and after cell culture work
- do not handle more than one cell line at a time
- do not share media or other reagents among different cell lines
- The laminar should be on 30 minutes before you start working.
- Prepare **aliquots** of media, HBSS, enzymes and other buffers in 50 mL tubes to avoid contamination of stock solutions
- All media/reagent bottles and aliquots have to be **labelled with name and date**!
- Wipe laminar and every item you put in the laminar with **70% ethanol** or **Bacillol AFTissues** before and after working
- Don´t leave anything additional in the laminar
- All the bottles, pipette cases and other vials used in cell culture should be **opened in the laminar only** and resealed with Tixo straps after use
- Bottles and their tops must be disinfected
- Always **close bottles**, tubes an flasks between working steps
- **Do not reach over open flasks!**

2. Media and reagents for human placental endothelial cells

PromoCell + pregnant serum:
Endothelial Cell Basal Medium MV + Supplement Pack without FCS + **10% Pregnant Serum** (collected from hormone laboratory)
Prepare 250 mL, sterifiltration, make 50 mL aliquots
Cave: for fresh isolation of fpECs only!!!
And for media change in 12 well plates before 1st Passaging step!

Pregnant Serum:
Collected from hormone laboratory staff
Pool serum and heat inactivate for 30min at 53°C in wather bath
Make 50 mL aliquots
(Storage: LaborI -20°C, bottom drawer and -40°C)

PromoCell - Media:
Endothelial Cell Growth Medium MV Kit (Media + Supplement Pack), **#C-22120** PromoCell, Distrubutor: Biomedica
Endothelial Cell Growth Medium MV, Basal media bottles only, 500 mL **# C-22220**
Endotheliall Cell Growth Medium MV SupplementPack, #C-39220 (Hydrocortisone, bovine hypothalamic extract, hEGF, 5% FCS)
Store Supplement Pack at -20°C
Store Media at +4°C in cooling room in the dark

PromoCell ++:
PromoCell MV + Supplements + 5% FCS **+ 500µl Gentamicin**
Prepare a 500 mL bottle, add new sticker on the bottle including date of preparation and name
Only prewarm aliquoted media at 37°, because the supplements are temperature sensitive!
Media bottle can be stored at room temperature during work
Use:

- General cultivation for established endothelial cells!

PromoCell +:
PromoCell MV +5% FCS **+ 500µl Gentamicin** WITHOUT Supplements
Use:

- For experiments with fpECs in which supplements might influence the results!

PromoCell w/o:
PromoCell MV WITHOUT FCS WITHOUT Supplements
Use:

- For starving cells before special compound testing experiments
- For preparation of Freezing media

1x HBSS:
500 mL bottle, #14175053, Gibco

- For all washing steps

Cell culture Flasks- Vent Cap:
75cm^2: Nunc Easy Flask, #156499
25cm^2: Nunc Easy Flask, #15636

Endothelial cells need to be cultivated on Gelatine coated flasks!

<u>Prepare 2% Gelatine stock solution</u>
using porcine skin gelatin #G9391-100g, Sigma-Aldrich

- **weigh 10g** of porcine skin gelatin and transfer into a 500 mL glas bottle
- ad **500 mL of 1x HBSS**, #14175053, Gibco
- autoklave Gelatine solution
- let cool down, keep in water bath at 37°C
- add 10 mL Gentamicin (2%), #15750037, 50mg/ mL, Gibco
- aliquot in 50 mL Falcons, store at +4°C
- prior to coating: dilute 2% Gelatine 1:2 using 1x HBSS. End concentration is **1% Gelatine**!

Coating:

- For 75cm2 Flasks add ~10 mL of 1% Gelatine solution per flask
- Agitate so that the bottom of the flaks is coated, and transfer gelatin to the next flask and so on.
- Keep flaks standing in an upright position, allowing the remaining liquid to float down.
- Aspirate the liquid
- Store flasks in a humid incubator at 37°C to let the gelatine dry for at least 1h
- Minimum time is 30- 45min!
- Before use check if some liquid is still remaining.

Coated plastic ware can be used up to 1 week!
Before starting cell culture work make sure, that you have enough coated flaks.
If you take coated flasks from any other person to save time, coat new ones!

Collagenase/ Dispase:

- 500mg, Roche # 11097113001
- Dissolve 1 vial in 5 mL HBSS (take volume out of 500 mL 1x HBSS bottle)
- add dissolved enzyme to 500 mL 1x HBSS (Conc. of 1mg/ mL)
- add 10 mL Pen/Strep (2%)
- sterilfiltrate
- prepare 50 mL aliquots and store at -20°C
- Prior to use for isolation of fpECs thaw at 37°C and dilute 1:2 in 1x HBSS (0,5mg/ mL including 1% Pen/Strep)

Human platelet lysate (hPL):

Platelet lysate is used for fresh isolations only!
It's obtained from the "Blutbank" in cooperation with Prof Schlenke
Kontakt: Dr. Susanne Macher (phone 81298)
Storage: -40°C, Gruppe 4, Tür 2, drawer 4 in the front „Jasmin reagents"

Important: hPL has to be centrifuged at 3700rpm for 30min prior to use!

This can repeated when centrifuged aliquots are again frozen.

PromoCell++ with 10% hPL

1. Thaw 1x50 mL hPL
2. Centrifuge at **3700rpm for 30min**
3. Filter supernatant through a **70µm cell strainer**
4. **Add 25 mL hPL to 250 mL PromoCell** media
5. Add supplements: Hydrocortisone, Gentamicin, hEGF, bovine hypothalamic extract
6. **Add 0.5 mL Heparin** Gilvasan 5000 I.E./ mL in order to avoid clotting of hPL in the media (**1:500, 10 I.E/ mL final concentration**)
7. Filter through a 250 mL sterile filter bottle
   In case of any hPL clots occurring in the media, repeat step 7!
8. Thaw remaining hPL again
   Use:
   - for fresh isolation of fpECs only!
   - And for media change in 12 well plates before 1. Passaging step!

PromoCell ++ with 5% hPL

1. Thaw 1x 50 mL hPL
2. Centrifuge at **3700rpm for 30min**
3. Filter supernatant through a **70µm cell strainer**
4. **Add 12.5 mL hPL to 250 mL PromoCell** media
9. Add Supplements: Hydrocortisone, Gentamicin, hEGF, bovine hypothalamic extract
5. **Add 0.5 mL Heparin** Gilvasan 5000 I.E./ mL in order to avoid clotting of hPL in the media (**1:500, 10 I.E/ mL final concentration**)
6. Filter through a 250 mL sterile filter bottle
   In case of any hPL clots occurring in the media, repeat step 7!
7. Thaw remaining hPL again
   Use:
   - Fresh isolations with initial cultivation in 10% hPL are cultivated in 5% hPL after 1st passaging step! (in T12)
   -

In case only small amounts of hPL media is needed for fresh isolations, only prepare 250 mL of each media.

3. Isolation of Feto Placental Endothelial Cells (fpEC)

Material :

- Latex gloves
- Sterile scissors, sterile forceps
- thread (Prolene, #883H, 75cm, 36 pieces/package, Ethicon)
- sterile gauze
- 50 mL perfusor syringe with needle (#8728810F-06, 20 pieces)
- 10 mL syringe
- Infusion canula (Optiva 2 I.V. Catheter Radiopaque, #1018, 18G, 1.30mm diameter, 50 pieces/ package, Smiths Medical) or
- Vasofix Safety Pur 20G, 1.1mm, #4269110S-01, Braun
- Sterile 150 mm Petri Dishes, Nunc #168381
- ß-Isodona (to disinfect)
- 40 mL FCS Defined or Non-Defined
- 1x HBSS (Gibco, #14175-053)

Media for microvascular endothelial cells:

PromoCell + pregnant serum:
Endothelial Cell Basal Medium MV + Supplement Pack without FCS + **10% Pregnant Serum** (heat inactivate prior to use)

For **fresh** isolations!

Gelatine coating:

Coat at least 1 hour before isolation a 12 well plate with 1% gelatine.

[porcine skin gelatine #69391-100g, Sigma-Aldrich, dissolved in 1x HBSS]

Enzyme solution:

One vial of Collagenase/Dispase (Roche, Cat: 11097113001, 500mg) is dissolved in 5 mL of 1x HBSS and added to 495 mL 1x HBSS, which gives 1mg/ mL.

10 mL of Penicillin/Streptomycin (10000µg/ mL) are added to the prepared enzyme solution, results in 200µg/ mL final concentration.

Sterile filter the enzyme solution, aliquot into 50 mL and freeze at -20°C.

Isolation Procedure:

- If there is a delay or birth during the night store the placenta at +4°C
- Dilute Collagenase/Dispase solution 1:2 to 0,5 mg/ mL in 1xHBSS
- Prewarm Collagenase/Dispase solution and PromoCell MV + Supplements + **10% Pregnant Serum Media** at 37°C
- Thaw 50 mL of FBS. Label 3x 50 mL Falcon tubes for each vessel (A1, A2, V1, V2)
- Add 20 mL of HBSS into 2 tubes for each vessel and 10 mL of FCS in 1 tube/ vessel

1. Use sterile gauze to disinfect the placenta with Betaisadona
2. Remove the amnion and cut off the vessels
    - Choose 1 to 3 mm diameter chorionic plate vein and 1- 2 mm chorionic plate artery (arteries are crossing the veins and are more tailed)
    - The minimum length of the vessel should be 3 cm, preferably as long as possible
3. Transfer the vessel to the first HBSS washing tube
4. Continue with the dissection of the other vessels. Following dissection transfer each vessel to its corresponding second HBSS wash tube.
5. Transfer vessel onto a sterile petri dish
6. Smooth out the vessel with the cannula to remove blood and to locate the lumen
7. Pull back the needle ~ 5mm from the tip of the plastic
8. Cannulate the vessel
9. As soon as the cannula is in the vessel, remove the needle to avoid any damage
10. Fix the vessel on the cannula with a thread

    Caution: this is a critical step! Avoid slippage of the vessel from the cannula!
11. Carefully flush vessel with 5 mL of 1x HBSS to eliminate blood and to get rid of ECFCs
12. Fill the syringe with 20 mL of prewarmed Collagenase/Dispase solution.
13. Remove air bubbles from the syringe
14. Connect the cannula onto the syringe
15. Start cell digestion, with the first 3 drops going to waste
16. Subsequent cell digest solution is collected into the tube containing 10 mL FCS

    Caution: Avoid touching the inner wall of the tube with the vessel, cause this might be a reason for contamination with fibroblasts
17. 20 mL enzyme solution is rinsed through the vessel for 6-8 min (5 mL/2min)
18. Canulate and digest all the vessels in the same way.

Using Syringe Pump:

Diameter of 50 mL syringe: **27,6mm**

Flow Rate: **2,5 mL/min**

19. Invert and centrifuge the digested suspension at 900rpm (200g) for 7 min at RT
20. Aspirate the supernatant and resuspend the pellet in 1 mL of PromoCell + Supplements + **10% Pregnant Serum** and transfer cell suspension into a well of a gelatin coated 12well plate
21. Incubate at 37°C at 12% oxygen
22. Perform media chance on **day 2 or 3 after isolation** with PromoCell + Supplements + **10% Pregnant Serum**
23. Observe cells daily and transfer them into a 12cm^2 or 25cm^2 flask when they reach up to 50% confluence
24. Upon this stage cells are cultivated in PromoCell + Supplements **+ 5% FBS**

Note:

After **2 – 4 weeks fpECs will grow** out and can be subcultivated. It takes about **another 4 weeks** till cells are expanded to 2 x 75cm^2 flaks and can be frozen for further use and seeded for Immunocytochemistry quality check on chamberslides!

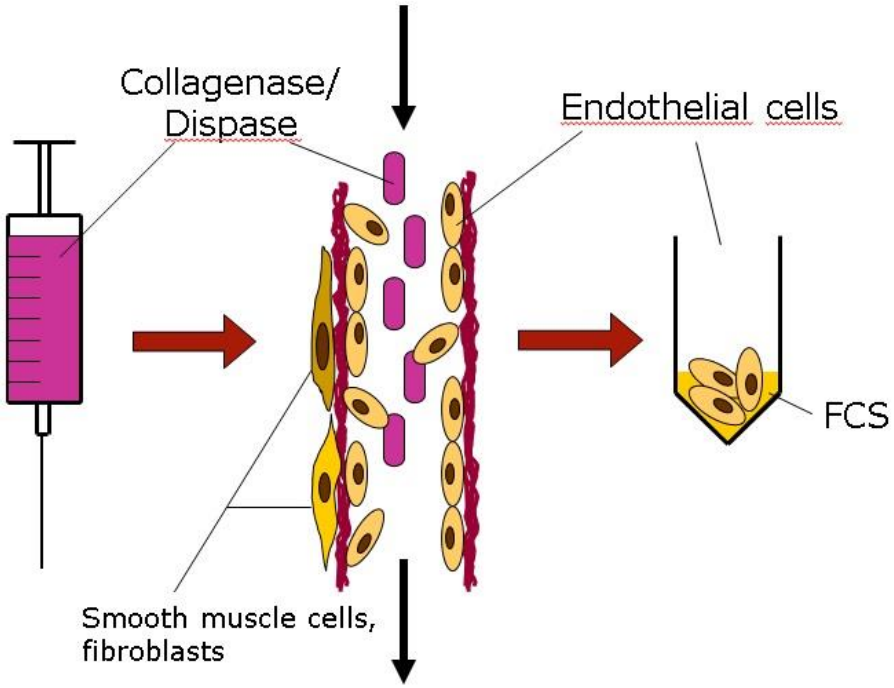
Till expansion to 75 cm^2 **Accutase** (Sigma) is used instead of TrypLe, to avaoid any damage of endothelial cell surface

Total time for establishing a new fpEC – Isolation: **2 months!**

After cells have been splitted for the 1st time, arterial and venous fpECs are separated and cultivated at different oxygen concentrations:

- **ECA:** 12 % oxygen (right incubator)
- **ECV:** 21% oxygen (left incubator)

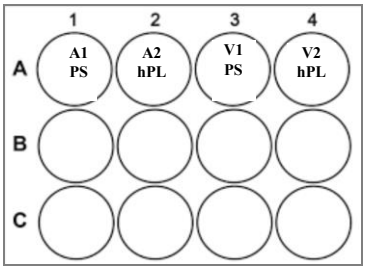
Isolation principle:



4. Subcultivation of hPECs (by Barbara Leopold)

4 vessels (2 x artery, 2 x vein) per placenta are excised and cultivated in

- PromoCell + Supplements + 10 % Pregnant Serum (PS) **or**
- PromoCell + Supplements + 10 % Platelet Lysate (hPL)



Perform medium change on day 2 or 3 after isolation, and then twice a week!

Observe cells daily and after reaching an adequate density transfer them into a collagen-precoated 12,5 cm² flask = **passage 1a**

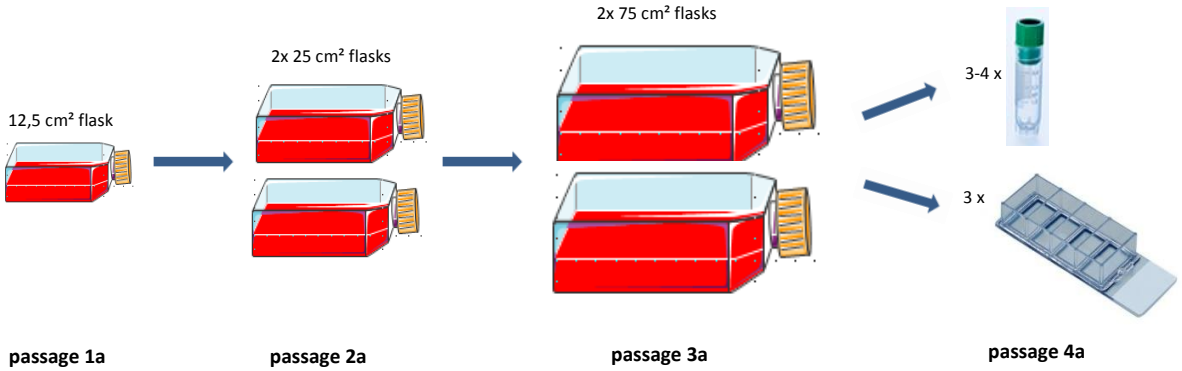
- remove medium
- wash with 1 mL HBSS
- add 300 µl Accutase
- incubate 3 min at 37 °C
- stop enzymatic activity with 1 mL medium and transfer to the prepared 12,5 cm² flask

At this stage, medium is changed:

- PromoCell + Supplements + 10 % Pregnant Serum (PS) → **PromoCell + Supplements + 5 % FBS (= PROMOCELL ++)**
- PromoCell + Supplements + 10 % Platelet Lysate (hPL) → **PromoCell + Supplements + 5 % hPL**

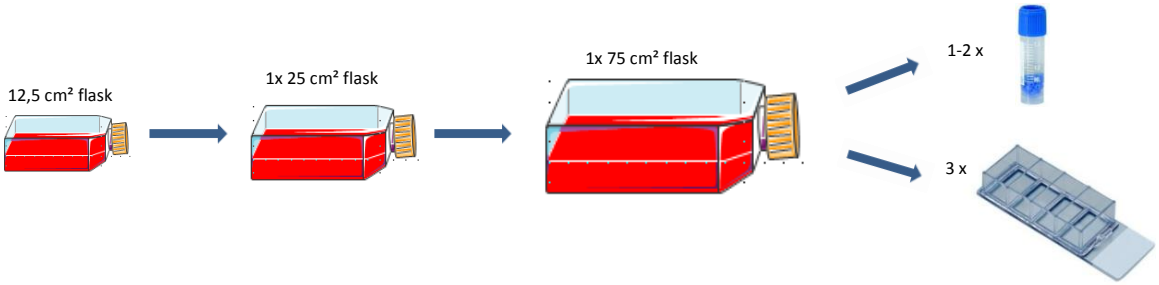
1. ECAs:

- After achieving confluence passage cells 1:4 into **two** 25 cm² flasks (**= passage 2a)**.
  Use 500 µL Accutase per flask.
- Each flasks is then further splitted 1:3 in a 75 cm² (**=passage 3a).**
  Use 1 mL Accutase per flask.
- Afterwards, the cells of the obtained two 75 cm² flasks are frozen and seeded in chamberslides for quality control:
  - 5 x 10^4 cells/well are seeded in three 4-well-chamberslides
    (12 wells in total → 6 x 10^5 cells)
  - Remaining cells are frozen in 3-4 cryovials with 1 mL freezing medium/vial.
    Label vials with **passage 4a.**



ECVs:

- After achieving confluence passage cells 1:2 into **one** 25 cm² flask (**= passage 2a)**.
  Use 500 µL Accutase per flask.
- This flask is then further splitted 1:3 in a 75 cm² (**=passage 3a).**
  Use 1 mL Accutase per flask.
- Afterwards, the cells of the obtained two 75 cm² flasks are frozen and seeded in chamberslides for quality control:
  - 5 x 10^4 cells/well are seeded in three 4-well-chamberslides
    (12 wells in total → 6 x 10^5 cells)
  - Remaining cells are frozen in 1-2 cryovials with 1 mL freezing medium/vial.
    Label vials with **passage 4a.**



5. Splitting of fpECs

1. aspirate the media of a 75cm^2 flask containing ECAs or ECVs
2. wash cells with 10 mL 1x HBSS (Gibco, #14175-053)
3. add 1,5 mL of 1x TrypLE Select (Gibco, # 12563011) (or 2 mL Accutase) and incubate for 3 -5 min at 37°C
4. resuspend cells in 9 mL PROMOCELL++ to inactivate the enzyme (10 mL suspension in total)
5. [Optional: centrifuge cells at 800 RPM for 4min at 4°C to remove Trypsin, resupend in 10 mL PROMOCELL++]
6. Divide cells into different flaks for cell expansion (depending on splitting ratio)
7. Fill up 75cm^2 flask to 10 mL with PROMOCELL ++ media
8. [Optional: Change media after 4-5 hrs when cells have attached or on the following day to eliminate TrypLE]

Splitting ratio:

- **ECA: 1:3** in the beginning, standard rate is **1:4**, maximum rate up to 1:7 depending on growing rate of isolation
- **Note: after thawing of ECs, it is mandatory to split them 1:3 and a minimum of 1 flask must be frozen to keep the cryo stock in low passages!**
- **ECV: 1:2 – maximum of 1:4**, ECVs are growing very slowly!

TrypLE and Accutase volumes

Accutase (Sigma-Aldrich, A6964-500ML) is more gently to the cells and therefore higher volumes compared to TrypLE are required. Cells can get incubated with Accutase up to 20min and Media-FCS inactivation is not necessary.

Culture vessel	TrypLE volume [mL]	Accutase volume [mL]
12 well plate	0.3	0.3
6 well plate	0.5	0.5
12.5 cm2 flask	0.5	0.5 - 1
25 cm2 flask	1	1 - 1.5
75 cm2 flask	1.5	2

6. Freezing of fpECs:

Prepare Freezing Media:		
35 mL PROMOCELL	(without Suplements)	(70%)
10 mL FCS	(Hyclone, defined)	(20%)
5 mL DMSO	(Sigma- Aldrich or Serva)	(10%)

Aliquot in 50 mL Falcon Tubes, store at +4°C for up to 4 weeks or prepare more aliquots and store at 20°C. In case of observing crystallization, discard freezing media

Freezing:

1. aspirate the media of a 75cm^2 flask containing ECAs or ECVs
2. wash cells with 10 mL 1x HBSS
3. add 1,5 mL of 1x TrypLE Select and incubate for 3 -5 min at 37°C
4. resuspend cells in 9 mL of **DMEM+ + (1% Pen/Strep, 10%FCS)** to inactivate the enzyme
5. Optional: count the cells using a CASY cell counter, add 100µl of cell suspension to 10 mL of Casy Ton (1:100)Use following cell concentrations for freezing:

ECAs: minimum of 1,5 x 10^6/vial
ECVs: minimum of 2 x 10^6/vial

Or if you don't count the cells divide cells as followed:
ECAs: 1xT75 into 2-3 freezing vials
2xT75 into 4-5 freezing vials, ect…

ECVs: 1xT75 into 1 freezing vial
3xT75 into 4 freezing vials

6. centrifuge cells at 800 rpm for 4min at 4°C (DMEM ++ gets eliminated)
7. Meanwhile print out tube labels and prepare cryo vials (**keep them on ice or in a cooling box**)
8. aspirate supernatant and resuspend cell pellet in **cold freezing media** (directly from ice or fridge), according to cell concentration (work on ice)
9. Use **1- 1,5 mL PROMOCELL freezing media** per freezing vial
10. Immediately transfer vials into a freezing container and store at -80°C
    a. If you use Mr. Frosty keep cells, freezing media and freezing vials on ice!
    Make sure that enough Isopropanol is in the container and that Mr. Frosty was precooled in the fridge or on ice!

b. If you use Cool Cell cells and freezing vials can be kept at room temperature, but use precooled freezing media from the fridge.

Make sure that the black ring is placed in the center of the cool cell and that it has room temperature!

If you use the Cool Cell coming directly from -80°C, prewarm the ring in the water bath prior to use!

11. Transfer cells for long term storage into liquid nitrogen after 4 - 24 hours

Labelling of vials:

ECA 159/ 7b
PromoCell
1,5 x 10^6
30.06.2018

Labelling of lid caps:

ECA
159

Color Code of vials:

ECA: green **ECV: blue**

Diabetic isolations: ECAd and ECVd yellow

7. Storage of fpECs in liquid nitrogen tank

We have 2 different N2 tanks:

- **N2-Tank Gyn-01: For placenta tissue and Trophoplasts, new cells from other labs that might are contaminated with Mycoplasm**

- **N2-Tank Gyn-02: For primary Endothelial Cells and Cell Lines (no tissue!)**

- ✓ All racks, boxes and samples are listed in

  "Register Stickstofftank_Tank xy_Ständer-xy lists" in:

  F:\Forschung\5_Stickstofftank & _Freezer\N2-Tank_1

  F:\Forschung\5_Stickstofftank & _Freezer\N2-Tank_2

- ✓ An overview of each tank is shown in:

  " Overview_N2 Tank 1.xls"

  " Overview_N2 Tank 2.xls"

- ✓ Keeping the order here is very strict and laborious!
- ✓ **Always delete the vials you have taken from the tank immediately in the Register files!**
- ✓ **Always record new vials you put in the boxes in the register immediately!**
- ✓ Everybody is responsible of transferring his/her own samples from the Cool Cell boxes into the N2 tanks!
- ✓ Transfer your cells from the Cool Cell boxes into the N2 tanks within 1 week

Thawing of fpECs- standard protocol:

1. Switch on the Thaw Star device (needs some time for initialisation)
2. Take out vials from the liquid nitrogen tank and place them in a small liquid nitrogen dewar
3. Pipet 10 mL of **DMEM++ (1% Pen/Strep, 10%FCS)** in a sterile 50 mL Falcon tube
4. Place a cold cryo vial into the Thaw Star and make sure that it's catched by the device
5. Note: If you thaw cells at 37°C in the water bath, you need to make sure that there is still a **small ice clump** remaining in the suspension (DMSO is cell toxic at room temperature!)
6. If you have more than 1 cell line, **only thaw one vial after another**!
7. Take out cell suspension with a 1000µl pipette and transfer it into the prepared 50 mL tube
8. Make sure that you don't generate too much air bubbles cause this will stress cells
9. Rinse vial once with 1000µl of fresh media to collect the remaining cells and transfer them also into the tube
10. Centrifuge cells at 800rpm for 4min
11. Aspirate the supernatant (Removal of DMSO)
12. Gently resuspend cells in 10 mL PROMOCELL++ and seed them in a T75 flask
13. Change media after 24hrs – if cells have attached!

Note:

Centrifugation of the cells at 800rpm for 4min at +4°C after thawing yields better cell growth, especially for ECVs!

ECVs can be cultivated up to 3 days without media change after thawing, because they need more time to get attached and to recover.

Cell passage determination

- Fresh isolations get frozen as an **"a"** according to freezing for the 1st time eg. ECA 159 4a
- When thawed the next time it becomes a ECA 159 "4b"
- The **letter "b"** indicates that cells have been **thawed once**
- **Passage number** increases with each trypsinisation (splitting) step eg. 4a => 5a
- If you have ECA 159 6a and you trypsinize them for freezing, they get frozen as 7a and thawed as 7b!
- **It is not allowed to thaw cells with passages below 6**!

- Passages 3-5 have to be kept as a backup for our cell stock! Passage 3 is the 1st passage we are able to freeze.
- **Never use the last vial of cells with low passage and low freezing number!**
- Use higher passage number of cells for routine experiments, establishing of methods, Protein, DNA, ect…
- If isolations grow nicely, they can get used till passage 12-14
- Don't use cells that are reserved in the for different persons and/or projects N2 register!

More information can be found in F:\Forschung\10_Zellkultur

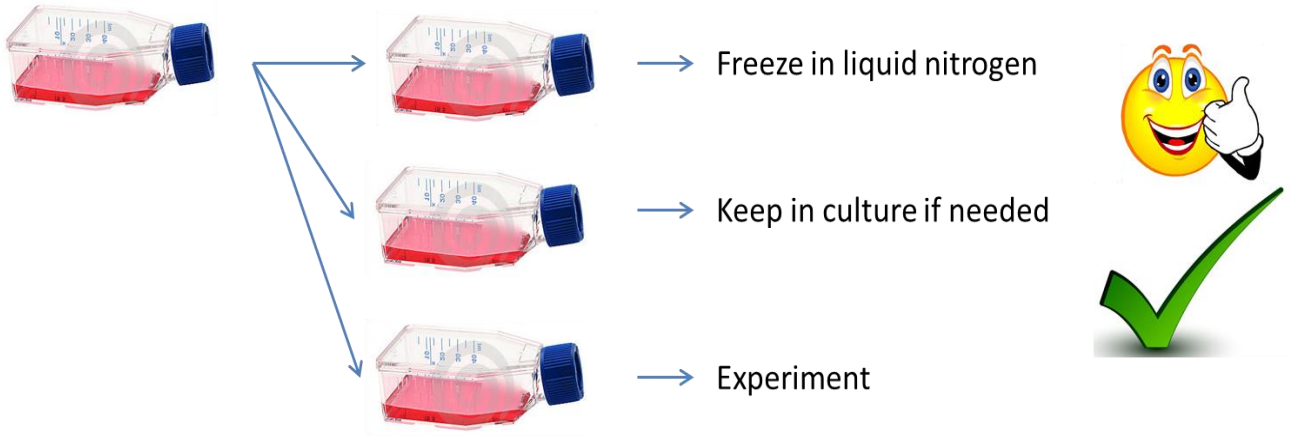
8. Important notes when planning your experiments and for freezing of fpECs

PLEASE SPLIT THE CELLS **BEFORE** YOUR EXPERIMENTS AND LEAVE ONE FLASK TO FREEZE IN A VIAL, THAT WAY WE WILL STILL HAVE CELLS WITH A PASSAGE NOT SO OLD

ESPECIALLY WITH THE DIABETIC CELLS!!

ISOLATION OF CELLS IS A LOT OF WORK AND IT TAKES APPROXIMATELY **2 MONTHS** TO HAVE A VIAL.

THEREFORE PLAN WELL YOUR WORKING TIMES AND YOUR EXPERIMENTS CONSIDERING 1 SPLITTING STEP MORE THAN USUAL.



7. Processing of cells for DNA-, RNA-, and protein isolation

Harvesting of fpECs for DNA isolation: DNA Mini Kit, Qiagen

1. Trypsinize cells using TrypLE (see protocol "**Splitting of fpECs"**)
2. Determine cell number on the Casy
3. Use **1x 102 - 5x106 cells/ isolation** (in 1 x 75cm^2 normally - **1,5 x106 – 3 x106** can be collected) Avoid using more than 5x106 cells/ isolation as this will cause clumping on the Qiagen columns
4. Centrifuge cells at 900 rpm for 4min in a 50 mL falcon tube, aspirate supernatant
5. Wash cells in 10 mL of **PBS** and centrifuge at 900 rpm for 4min, aspirate supernatant
6. Resuspend cells in **1000µl of PBS** at a maximum cell number of 5x106 cells and transfer in to a sterile autoclaved 1,5 mL tube
7. Centrifuge cells at 4000 rpm for 4min in a table top centrifuge, aspirate supernatant, vortex the pellet
8. Snap freeze the pellet by holding the tube in liquid N_2 for 3 times
9. Store at -20°C for further use
10. Isolate DNA using "DNA mini Kit" Qiagen and "Appendix B: Protocol for Cultured Cells"

Harvesting of fpECs for mRNA isolation: RNeasy Kit, Qiagen

1. Do not use more than **- 1x10^7 cells**
2. Detach cells either by trypsinization using TrypLE (see protocol "**Splitting of fpECs"**) or using a cell scraper
3. If you trypsinize cells wash the pellet at least once in PBS before adding buffer RLT
4. If you scrape cells wash the monolayer twice in 10 mL of PBS
5. **Prepare Buffer RLT**: add 10µl ß- Mercaptoethanol per 1 mL of Buffer RLT (stable up to 4 weeks at RT, but fresh preparation is recommended)
6. Resuspend cells in **appropriate** amount of Buffer RLT and transfer in to a sterile autoclaved 1,5 mL tube

Number of pelleted cells	µl Buffer	Dish diameter (6cm)	µl Buffer
< 5x10^6	350	< 6	350
5x10^6 - 1x10^7	600	**6-10**	600

7. Vortex cells properly and ensure that no cell clumps are visible

8. Store at -20°C for further use
9. Isolate RNA using "RNeasy mini Kit" Qiagen and Protocol: "Purification of total RNA from animal cells using spin technology" continue with step 3

Harvesting of fpECs for miRNA and mRNA isolation: miRNeasy Mini Kit, Qiagen

1. Always use a standardized cell number – eg. **$2x10^6$ cells**
2. Do not use more than **- $1x10^7$ cells –** it will clump the column
3. Detach cells either by trypsinization using TrypLE (see protocol "**Splitting of fpECs"**) or using a cell scraper
4. If you scrape cells wash the monolayer twice in 10 mL of PBS
5. If you trypsinize cells wash the pellet at least once with **10 mL PBS** before adding QIAzol and centrifuge at 800rpm for 4min at +4°C
6. Aspirate supernatant
7. Resuspend cells in **700 µl QIAzol** (collect pipet tips with QIAzol in a 50 mL tube and close it, in order to avoid vaporisation)
8. Vortex cells properly and ensure that no cell clumps are visible
9. **Store at -70°C** for further use

Isolate miRNA using "miRNeasy mini Kit" Qiagen and Protocol:"Purification of Total RNA, Including Small RNAs, from Animal Cells" continue with step 4.

Harvesting of fpECs for protein isolation: in RIPA Buffer

Preparation of RIPA Buffer:

Add 1 tablet of protease inhibitors (Roche) per 10 mL of RIPA Buffer (Sigma-Aldrich). Vortex a little bit and let the tablet dissolve (this takes about 30 min). Make aliquots of RIPA Buffer and store at -20 C° in the cell culture freezer. In general keep RIPA Buffer on ice.

1. Use 1x confluent T75 flask of ECs for protein isolation
2. Detach cells either by trypsinization using TrypLE (see protocol "**Splitting of fpECs"**) or using a cell scraper
3. If you trypsinize cells wash the pellet at least once in HBSS before adding **RIPA Buffer**
4. If you scrape cells wash the monolayer twice in 10 mL of HBSS
5. Resuspend cells in 100 µl RIPA for 75 cm^2 flasks, 50 µl RIPA for 25cm^2 flask, 70 µl RIPA for a well of a 6-well plate (when using cell scraper)

Reduce the volume of RIPA buffer accordingly if a higher protein concentration is required

For high protein concentration even resuspend cells in 100 µl RIPA for 2x75 cm² flasks!

6. Transfer samples to**1.5 mL tubes** , vortex
7. Store at -20°C for further use
8. Isolate protein according to the SOP “Preparation of protein lysates of human placental tissue and cells”

Important cell culture rules:

- ✓ If you used the last PROMOCELL++ media prepared → Prepare more!
- ✓ Don't forget your media aliquots in the fridge- use them all! The media is very expensive! (500 mL of PROMOCELL++ cost about 150€)
- ✓ All TUBES should be labeled with **CONTENT, DATE, and NAME** of person
    - All non labelled tubes will be discarded each Friday during cell culture cleaning!
- ✓ If the bottle for the vacuum pump is FULL → Empty it and add 70% EtOH to it
- ✓ If you used all the pipettes, Pasteur Pipets, Tips, Falcon, Flasks…. → Bring new ones from the storage room.

Freezing cells:

- ✓ **Don't** forget about your cells in the incubator! If they are too confluent, freeze them or split/ expand them for later freezing
- ✓ **Never discard cells!**
    - Ask group members if they need some
    - Always freeze them in a vial
    - or freeze them for Protein-, DNA- RNA isolation
- ✓ - If you used the last prepared freezing media → Prepare more.
- ✓ - The entire freezing process ENDS after you move the vials from Mr. Frosty to liquid Nitrogen

Please do NOT forget the vials in Mr. Frosty!!

- ✓ After working in the bench, clean the surface, and if you spilled some liquid, also clean under work plate using 70% EtoH or Bacillol!
- ✓ In the afternoon always ask if someone else is going to use the bench, before shutting down cell culture

<u>If you are the last one working:</u>

- ✓ - close the bench/es after cleaning
- ✓ - put the bottles of media and buffers on the fridge
- ✓ - Turn off the water bath, microscopes, plate heater, casy counter (after cleaning it) and the computers

WE WORK TOGETHER AND IS OUR RESPONSIBILITY TO TAKE CARE OF OUR WORKING PLACE!

Exosome Isolation from endothelial cell culture supernatants

Materials/Reagents

For Cell Culture:

Material/Reagent	Supplier	Order No.
Endothelial Cell Growth Medium MV Kit	PromoCell	C-22120
Exosome depleted FBS	Gibco (Thermo Fisher)	A2720801
1xHBSS	Gibco	
DMEM	Gibco	
Pen/Strep		
Hyclone FCS		
Cell scraper	Corning	3010
RIPA buffer, Protease Inhibitor	Sigma Aldrich	R0278
Qiazol (a part of the miRNeasy Mini Kit)	Qiagen	217004
50 ml Falcon tubes	Falcon	
1.5 ml Eppendorf safe-lock tubes	Eppendorf	
2 ml Eppendorf safe-lock tubes	Eppendorf	

For Isolation Procedure:

Material/Reagent	Supplier	Order No.
PBS (PBS tablets)	Medicago	09-9400-100
Aqua dest.	Ampuwa, Fresenius Kabi	
Qiazol (a part of the miRNeasy Mini Kit)	Qiagen	217004
0.2 µm filter (Nalgene 25 mm Syringe Filter, Nylon, 0,2 µm pore)	Thermo Fisher Scientific	723-2520
0.02 µm filter (Anotop 25 mm Sterile Syringe Filters, 0.02 µm)	GE Healthcare Life Sciences	6809-2102
Millipore Steriflip 50 ml		
10 ml syringes ()		
5 ml syringes (Braun inject)	Braun	4606051
2 ml syringes (Braun inject)	Braun	4606027V
Sterican 21G x 4 ¾, 0.80 x 120 mm	Braun	466 5643
Pierce™ Protein Concentrator PES, 100K MWCO, 20-100 ml	Thermo Fisher Scientific	88537
Ultra-ClearTM Centrifuge Tubes, 25x89 mm, 38.5 ml	Beckman Coulter	344058
Quick-seal Centrifuge tubes, 16x76 mm	Beckman Coulter	342413
50 ml Falcon tubes	Falcon	
Stripettes (5 ml, 10 ml, …)		
Tips for pipettes		
1.5 ml Eppendorf safe-lock tubes	Eppendorf	
2 ml Eppendorf safe-lock tubes	Eppendorf	
Allegra® X-12R benchtop centrifuge	Beckman Coulter	
Optima XE-90 ultracentrifuge (incl. Type 70.1 Ti rotor)	Beckman Coulter	
Sealing device		

Sample preparation

Cell culture protocol: SOP_cell culture feto-placental endothelial cells.
Endothelial Cells (ECs) are thawed and expanded in **PromoCell ++** (ECAs) or PromoCell ++ with 5 % hPL (ECVs) under standard culture conditions (37 °C, 12 % or 21 %O_2, 5 % CO_2, humidified atmosphere). Prepare **3 x 175 cm²** (21-24 ml) or 7 x 75 cm² flasks **per isolation**, giving 70 ml of conditioned media.

- **Grow** cells to a confluence of approximately **70 %**. Wash monolayer with 1x HBSS and make sure to aspirate **all l**iquid before **changing media** to PromoCell containing:
  - 5 % exosome depleted FCS (Gibco, # A2720803)
  - hEGF
  - Hydrocortisone
  - Gentamycin
  - **NO** hypothalamic extract!!!

  (Some cells don't like this media change and stop growing)
- Challenge cells for **48 h** at 12 % O_2 (ECAs) or 21 % O_2 (ECVs) under standard culture conditions.
- **Collect conditioned medium** in 50 ml falcons (2x35 ml per isolation). Harvest the monolayer of one flask and do a **cell count** on the CASY-TT analyser, **note** amount of volume to calculate **total** cell number.

Additionally harvest 1 flask (175 cm², 4 ml Trypsin 20 ml DMEM++) or 2 flasks (75 cm², 1.5 ml Trypsin 8.5 ml DMEM++) of each isolation for:

1. **Protein isolation on ice**:

- collect media, add 10 ml HBSS
- aspirate HBSS completely, add 600 µl RIPA (+ protease inhibitor)
- dispatch cells using a cell scraper
- transfer RIPA-cell-suspensions into a fresh 1.5 ml tube
- store samples at -20 °C (Dunkelkammer-Babsi-Box *Cells in RIPA*)

OR use the harvested cells from the cell counting with CASY:

- centrifugate 800 x g for 5 min at 4 °C
- aspirate DMEM++
- wash with 10 ml HBSS
- centrifuge 800 x g for 5 min at 4 °C
- aspirate supernatant
- add 300 µl RIPA (+Protease Inhibitor) and transfer RIPA-cell-suspensions into a fresh 1,5 ml tube
- store at -20 °C (Dunkelkammer-Babsi-Box *Cells in RIPA*)

2. **RNA** (use a cooling rack):

- collect media
- add 10 ml HBSS
- aspirate HBSS
- add 1400 µl Qiazol directly onto the cell monolayer
- use cell scraper and dispatch cells
- transfer Qiazol-cell-suspension into a fresh 2 ml tubes
- store at -70 °C (Freezer 4, 3rd shelf, 4th Rack)

Exosome isolation procedure

Preparation:

- Sterile filter prepared 1x**PBS** with **0.02 µm** filters and 5 ml syringe into 1-2x50 ml falcons the same day or the day before isolation procedure.

- **Pre-cool** centrifuges, because all centrifugation steps are performed at **4 °C**.

Workflow:

1. Transfer cell culture supernatant into two 50 ml falcon tubes. Centrifuge the samples at **500 x g for 10 min** and **4 °C**. **Discard** the **pellet** containing cells and cell debris.

2. Transfer the supernatant into **fresh** 50 ml falcon tubes and centrifuge them at **2500 x g for 20 min** and 4 °C in the Allegra X12R benchtop centrifuge (Geräteraum). **Discard** the **pellet** containing the smaller cell fragments and apoptotic bodies.

3. *Use the SOP for Optima XE 90 ultracentrifuge.*

   Split the supernatant of one isolation into three ultracentrifuge tubes in **equal volumes** (about 23 ml, only two isolations per run possible) (Ultra-ClearTM Centrifuge Tubes, 25x89 mm, 38.5 ml, Beckman Coulter # **344058**). For the right balance in the UZ, scale the samples and add 1xPBS (0.02 µm) if needed. Close the tubes with the metal lids. Then centrifuge them in the Optima XE-90 **ultracentrifuge** at **12 000 x g** (=rcf) for **30 min 4°C** with selecting Type 70 Ti rotor. (clean the metal lids afterwards)

4. **Collect** supernatant into 50 ml falcons with 10 ml stripette, keep it at the centre the pellet should be on the side.

   The pellet contains the **microvesicle** population (potentially you can't see a pellet). **Pool** the pellets in 0.2 ml 1xPBS filtered (0.02 µm) and store them at **4°C** until further analysis.

5. **Filter** the **supernatant** through a **0.2 µm filter** (Millipore Steriflip with vacuum pump or Nalgene® Syringe Filter, 0.2 µm with 10 ml syringe) in order to remove any particles larger than 200 nm.

6. **Concentrate** the filtrate in a Pierce Protein Concentrator PES (100 000 MWCO) and centrifuge the samples in the benchtop centrifuge at **1800 x g** and 4 °C until a volume of approx. 4 ml is left (at first for 5 min and then adapt the time).

7. Pipette rest against filter membrane and then **transfer** the concentrate of the upper filter device into falcon tube. Also add 5 ml 1xPBS (0.02 µm) to the upper device and to collect all liquid. Afterward add the suspension into one or two (for PBS and RNA) **Ultracentrifuge tubes** (Quick-Seal, 12.5 ml #342413) with 5 ml syringe and needle

(Sterican 21G x 4 ¾, 0.80 x 120 mm). Fill it carefully up with 1xPBS (0.02 µm filtered) to a volume of 12.5 ml without bubbles.
Seal it carefully with ……..

8. **Centrifuge** for **22 hours at 100 000 x g** and 4 °C in the **ultracentrifuge** with the Type 70.1 Ti rotor. (Start 13-14:00)
9. Use a scissor and **cut** an opening into the Quick-Seal tubes.
10. **Remove** the supernatant with a 10 ml stripette, be careful and hold it in the center – pellet should be on the side. **Resuspend** the pellet containing the exosomes in **250 µl** 1x PBS (0.02 µm filtered).

    For **miRNA**: Resuspend pellet in 700 µl Qiazol (pellet gets visible)
11. Determine protein concentration by **BCA** assay. (Use the SOP_BCA)
12. Determine size distribution using the **Nanosight** NS300 instrument. For the measurement dilute samples **1:50** in 1xPBS (0.02 µm). Additionally, measure a blank with 1xPBS (0.02 µm).
    Use 2 ml tube and dilute in total 1 ml (20 µl sample+980 µl filtered PBS).
13. **Snap freeze** exosome samples in liquid nitrogen and store at **-80 °C**.
    Or use them for further experiments, immediately.

Characterization

Characterization of isolated exosomes is done by **western blotting.**
Positive Marker: Syntenin, ALIX, **TSG101**
Negative Marker: Grp94, ApoA1 and ApoB

Antibody	Distributor/Order-No.	Dilution	Conditions	Size	2.Antibody
Syntenin	Abcam ab19903	1:750	ON 4 °C	32 kDa	antiRabbit 1:2000
ALIX	Covalab pab0204	1:500	ON 4 °C	91-93 kDa	antiRabbit 1:2000
TSG101	Abcam ab125011	1:1000	ON 4 °C	44 kDa	antiRabbit 1:2000
Grp94	Santa cruz, sz- 393402	1:1000	ON 4 °C	94 kDa	antiMouse 1:2000
ApoA1	Santa cruz, sc-58230	1:1000	ON 4 °C	28 kDa	antiMouse 1:2000
Apo B	Abcam, ab39560	1:1000	ON 4 °C	260/516 kDa	antiMouse 1:2000

Standard Operating Procedure

Pierce BCA™ Protein Assay Kit

ThermoScientific #23227

For protein assays using Bicinchoninic Acid

BCA Protein Assay Reagent Kit, sufficient reagents for 250 test tube or 2,500 microplate assays

Kit Contents:

23228 BCA Reagent A, 500 ml, containing sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.1 M sodium hydroxide (Product No. 23225 contains 2 x Product No. 23228)

23224 BCA Reagent B, 25 ml, containing 4% cupric sulfate

23209 Albumin Standard Ampules, 2 mg/ml, 10 x 1 ml ampules containing bovine serum albumin (BSA) at a concentration of 2.0 mg/ml in 0.9% saline and 0.05% sodium azide

Kit storage at room temperature.

Equipment not supplied by the manufacturer:

37°C incubator

96 well Microtiter plate flat bottom (Nunc)

Multiwell plate sealer (Sigma A5596-100EA)

Microplate reader

Introduction:

The Pierce BCA Protein Assay is a detergent-compatible formulation based on **bicinchoninic acid (BCA**) for the colorimetric detection and quantitation of total protein. This method combines the well-known reduction of Cu+2 to Cu+1 by protein in an alkaline medium (the biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu+1) using a unique reagent containing bicinchoninic acid.

The purple-colored reaction product of this assay is formed by the chelation of two molecules of BCA with one cuprous ion. This water-soluble complex exhibits a strong **absorbance at 562 nm** that is nearly linear with increasing protein concentrations over a broad working range (20–2,000 µg/ml).

Accordingly, protein concentrations generally are determined and reported with reference to standards of a common protein such as bovine serum albumin (BSA).
A series of dilutions of known concentration are prepared from the protein and assayed alongside the unknown(s) before the concentration of each unknown is determined based on the standard curve.

Preparation of Standards and Working Reagent

Preparation of Diluted Albumin (BSA) Standards

Use Table 1 as a guide to prepare a set of protein standards. Dilute the contents of one Albumin Standard (BSA) ampule into several clean vials, preferably using the same diluent as the sample(s).

Table 1: Preparation of Diluted Albumin (BSA) Standards

Dilution Scheme for Microplate Procedure (Working Range = 20–2,000 µg/ml)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
	µl	µl	µl/ml
A	0	300 µl of Stock	2000
B	125	375 µl of Stock	1500
C	325	325 µl of Stock	1000
D	175	175 µl of vial B dilution	750
E	325	325 µl of vial C dilution	500
F	325	325 µl of vial E dilution	250
G	325	325 µl of vial F dilution	125
H	400	0	0=blank

Preparation of the BCA Working Reagent (WR)

Use the following formula to determine the total volume of WR required:

(# standards + # unknowns) x (# replicates) x (volume of WR per sample) = total volume WR required

Example: for the microplate protocol with 3 unknowns and 2 replicates of each sample:
(9 standards + 3 unknowns) x (2 replicates) x (200µl) = 4,8 ml WR required

Prepare WR by mixing **50 parts of BCA Reagent A with 1 part of BCA Reagent** B (50:1, Reagent A:B).

For the above example, combine 5 ml of Reagent A with 0,1 ml of Reagent B.

Note: When Reagent B is first added to Reagent A, a turbidity is observed that quickly disappears upon mixing to yield aclear, green WR. Prepare sufficient volume of WR based on the number of samples to be assayed. The WR is stable for several days when stored in a closed container at room temperature (RT).

Microplate Procedure

Pipette **5 µl of each standard or unknown sample replicate** into a well of a 96 well microplate

Add **200 µl of the WR** to each well and mix plate thoroughly on a plate shaker for 30 seconds.

Cover plate with a **Multiwell plate sealer** and incubate in a 37°C incubator at **37°C for 30 minutes**.

Cool plate to RT.

Measure the **absorbance at 562 nm** on a plate reader.

Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562 nm measurements of all other individual standard and unknown sample replicates.

Prepare a standard curve by plotting the average Blank-corrected 562 nm measurement for each BSA standard vs. Ist concentration in µg/ml. Use the standard curve to determine the protein concentration of each unknown sample.

Standard Operating Procedure

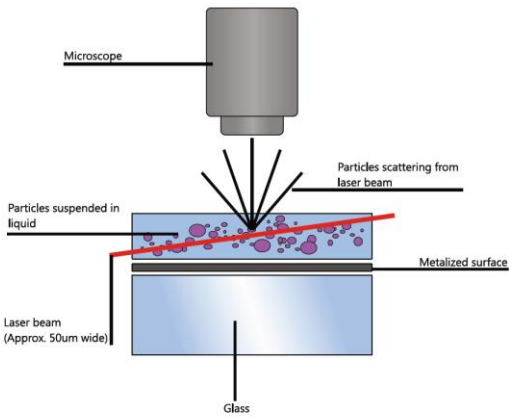
Messung am Nanosight NS300

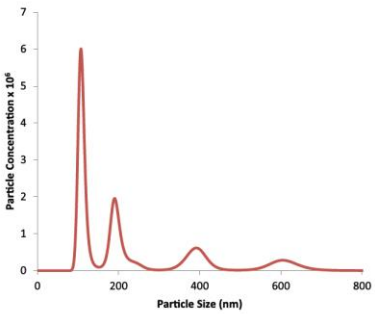
Prinzip:

Der NanoSight NS300 ist ein Laser-basierendes System, das die automatisierte Analyse der Größenverteilung und Konzentration aller Typen von Nanopartikeln im Größenbereich von 10 nm bis 200 nm Durchmesser ermöglicht.

Das Gerät verwendet die Nanoparticle Tracking Analysis (NTA) Technologie, die die beiden Eigenschaften Lichtstreuung und Brownsche Molekularbewegung nutzt, um die Größenverteilung und die Partikelkonzentration in Suspension zu ermitteln.

Ein Laserstrahl wird durch die Probenkammer geleitet und die Partikel in Suspension, die sich in der Bahn dieses Strahls befinden, streuen das Licht so, dass sie durch ein Mikroskop mit 20-facher Vergrößerung, auf dem eine Kamera montiert ist, visualisiert werden können. Die Kamera arbeitet mit 30 Bildern pro Sekunde und erzeugt eine Videodatei der Partikel, die sich in Brownscher Molekularbewegung bewegen. Die Software verfolgt die Partikel einzeln und berechnet mit Hilfe der Stokes-Einstein-Gleichung ihre hydrodynamischen Durchmesser.





Graph of a sample with distinct peaks at 100 nm, 200 nm, 400 nm and 600 nm.

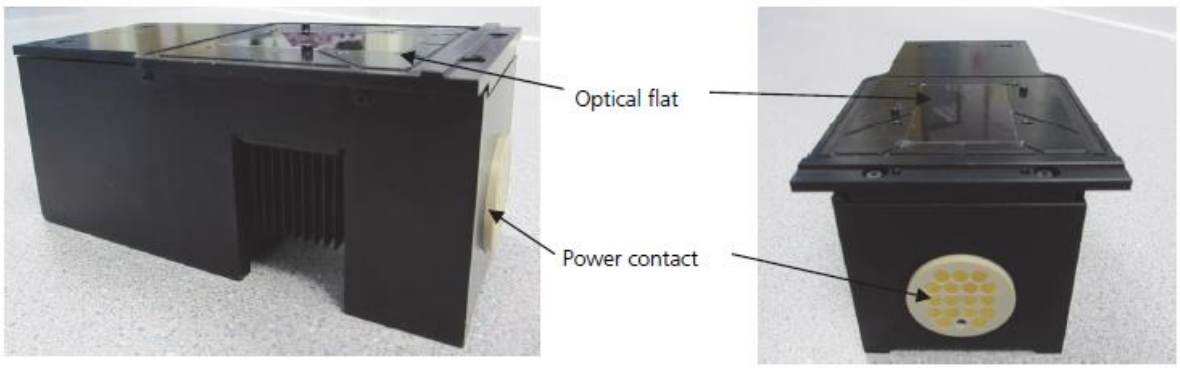
Material:

Laser-Modul: 488 nm oder 405 nm
Spritzenpumpe
Flow-cell Top-plate oder O-ring Top-plate
4 Befestigungsschrauben
Imbussschlüssel für Flow-cell Top-plate
1 ml-Feindosierungsspritze
A.d.-Spritzflasche
10% Ethanol-Spritzflasche
Fuselfreie, dünne Tücher

Messung am NanoSight:

Zuerst Gerät, einschalten (seitlicher Kippschalter)
dann PC einschalten!

Laser-Module:

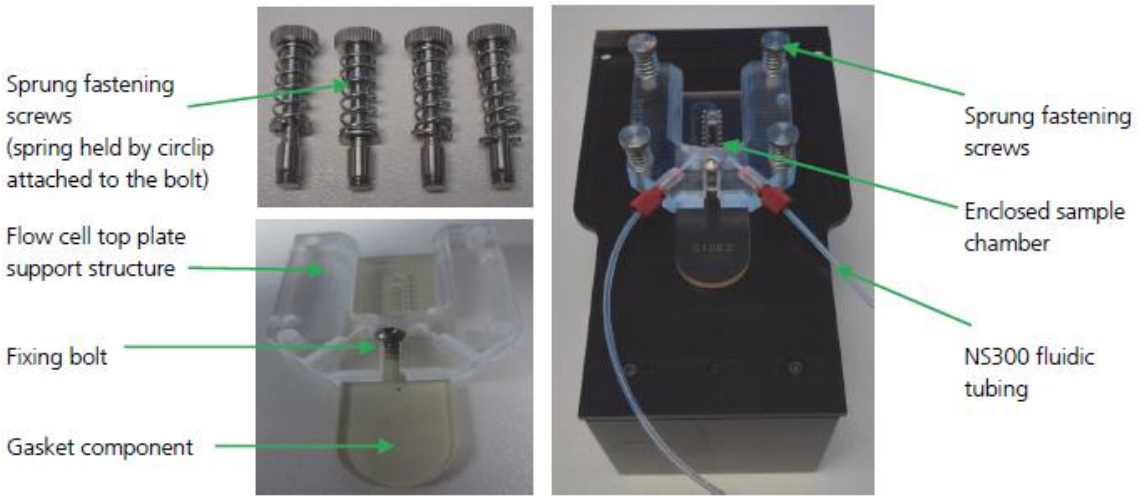


Flow-Cell Top-Plate:

Besteht aus einer Dichtungskomponente (=Gasket component) und einer Tragstruktur (Flow-cell top-plate support structure).

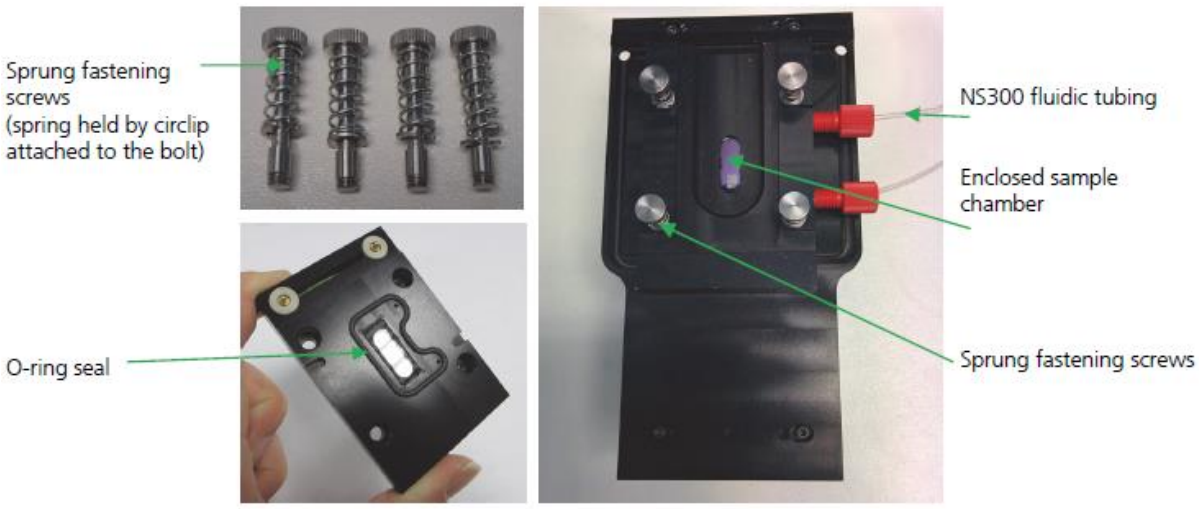
Dichtungskomponente mit Tragstruktur mit Hilfe des 2 mm Inbusschlüssels verschrauben und das Ganze mit den 4 Befestigungschrauben (Sprung fastening srews) auf der Glasplatte (optical flat) des Lasermoduls vorsichtig befestigen. Schrauben immer diagonal festschrauben, sonst könnte die Glasplatte brechen.

Flow cell Top-Plate



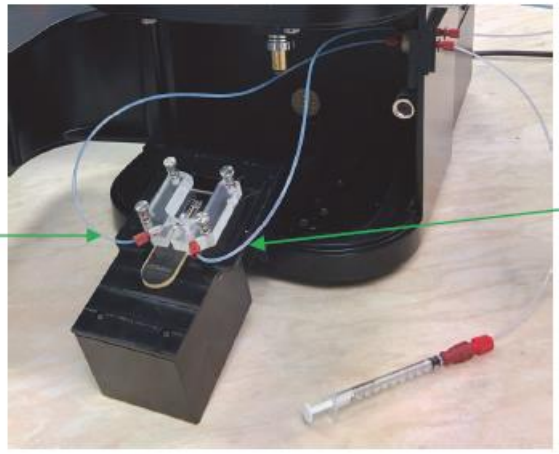
O-Ring Top-Plate:

Immer darauf achten, dass die O-Ring Dichtung in der Nut ist, sonst könnte die Glasplatte brechen!
O-Ring Top-Plate mit NS300 fluidic tubing (rechts) und der enclosed Sample chamber mit den 4 Befestigungsschrauben auf der Glasplatte befestigen.



Zu- bzw. Ableitung an der Innenseite des NS300 festschrauben:
Flow-Cell top-plate:
Linker Schlauch vorne, rechter Schlauch hinten am NS300 festschrauben
O-Ring Top-plate:
Milchigen vorderen Schlauch vorne, transparenten hinteren Schlauch hinten festschrauben

The tubing on the left-hand port on the top-plate should be attached to the left inlet port on the casing of the NS300.



The tubing on the right-hand port of the top-plate should be attached to the right waste port on the casing of the NS300.

The outlet tubing (wider bore) should be attached to the back port of the top-plate and to the right waste port on the casing of the NS300.

The inlet tubing (narrower bore) should be attached to the front port of the top-plate and to the left inlet port on the casing of the NS300.

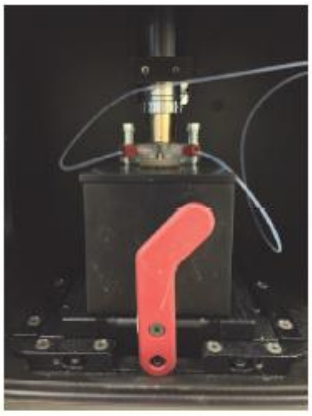
For instructions on how to assemble and clean the O-ring top plate see Section 2.2.4.2

Probe gut vortexen und entsprechend verdünnen mit filtriertem PBS (1:40-1:300).
Mit 1ml Feindosierungsspritze Probe aufziehen, an Schlauch stecken und manuell laden (ca. 0,5ml), dabei Laser-Modul herausnehmen und schräg halten.

Probe mit Spritze langsam, vorsichtig, luftblasenfrei laden, sodass die Flüssigkeit in die Top-Plate tritt.

Laser-Modul mit befestigter Flow-Cell Top-Plate oder O-Ring Top-Plate in das Gerät stellen und Hebel umlegen.

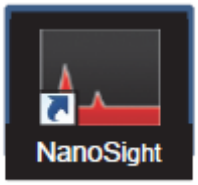
a) Laser module mounting / removal



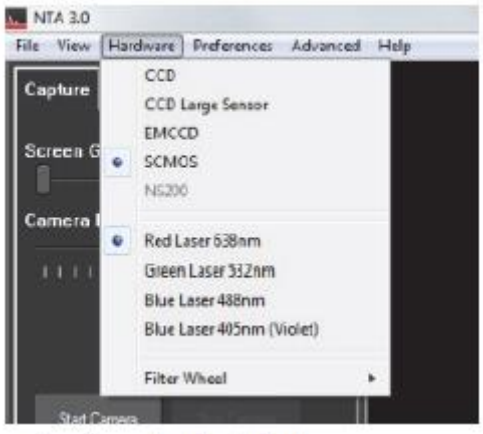
b) Laser module locked in position

Feindosierungsspritze in Spritzenpumpe einspannen, vorne und hinten einrasten und mit Schieber (Knopf auf der Seite hineindrücken zum Bewegen) fixieren, mit beiden Schrauben hinten festschrauben.

Am PC **Programm NTA 3.2** starten



Unter **Hardware** den gewünschten **Laser einstellen**:
Exosomen: Blue Laser 488nm



Kamera starten mit **Start camera**

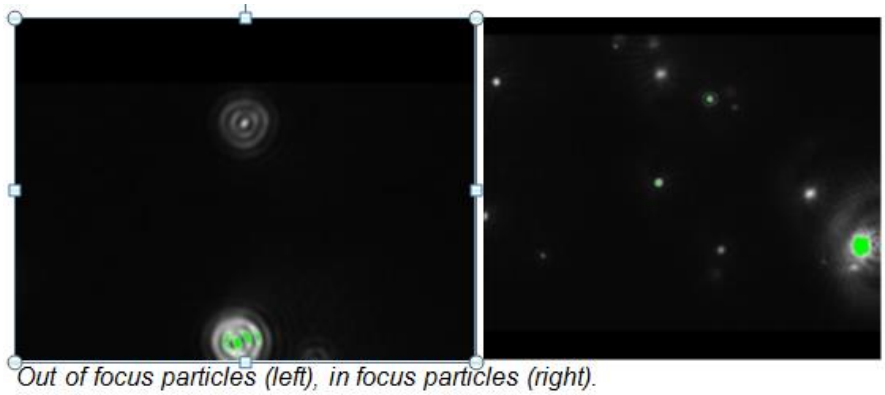
Capture anklicken links oben am Screen.

Kameraeinstellung (Camera level):

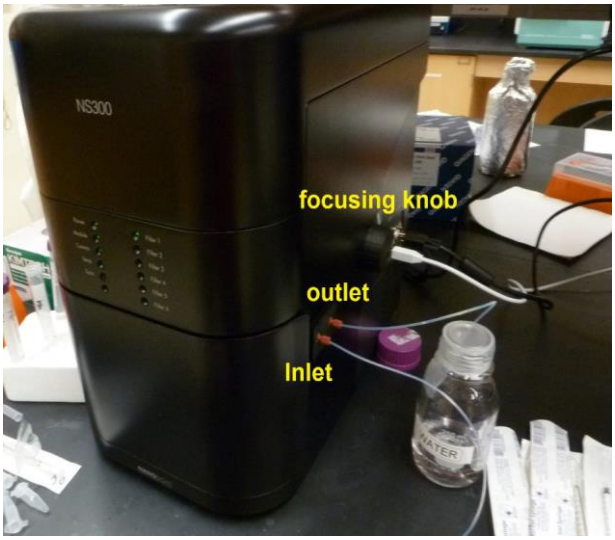
Kameralevel so wählen, dass nicht so stark leuchtende Partikel noch gut sichtbar sind, aber stark leuchtende Partikel nicht übersättigt sind.

Level einstellen entweder manuell oder
Auto Set up camera level only anklicken und auf **Auto Setup** klicken: Kameralevel wird von der Software automatisch eingestellt
Level 9-11 optimal

Fokuseinstellung entweder **manuell** mit Drehknopf rechts am Gerät oder mit der Software unter **Hardware/Focus** einstellen: Partikel sollen keine Ringe um sich haben.



Out of focus particles (left), in focus particles (right).



Setup a measurement:

Das **Measurement-Fenster** ist in der Mitte unten am Screen.
Den Reiter SOP anklicken
Die Software gibt 3 Auswahlmöglichkeiten:
Recent Measurement, wo man Messparameter eines früheren Experiments verwenden kann
Quick Measurement für eine Einzelmessung
Standard Measurement für mehrere Messungen

Standard Measurement Options:

Number of Captures: Anzahl der Videos festlegen.
Es wird empfohlen 3-5 Messungen pro Probe zu machen.

Capture duration: Dauer der Videos festlegen
Es wird eine Messzeit von 30-60 Sekunden empfohlen.

Temperature: wenn während der Messung eine konstante Temperatur unabhängig von der Umwelttemperatur erwünscht wird. Es kann ein Temperaturbereich zw. 20 und 50°C gewählt werden. Use Temperature Control + Target Temperature einstellen

Unter **Advanced settings** kann man die Viskosität und Verdünnung je nach Probe eingeben.

Unter **Advanced** kann man den kontinuierlichen Fluss der Spritzenpumpe einstellen:
Bei O-Ring Top-Plate: recommended flow speed 50-80
Bei Flow-cell Top-Plate: 20-50

Unter **Base Filename** Speicherort und Name des Experiments auswählen.
Create script - Create and Run script - Advanced (rechts unten am Screen**)**

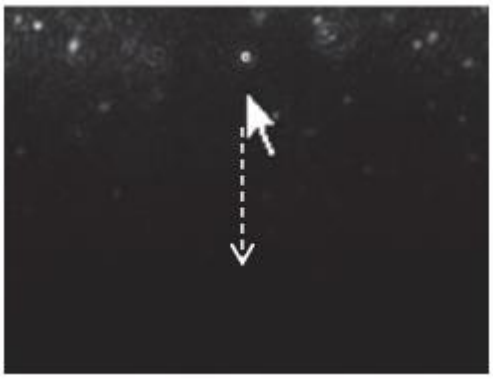
Wenn alle Parameter eingestellt sind, Messung starten mit **Create and Run Script**. OK.

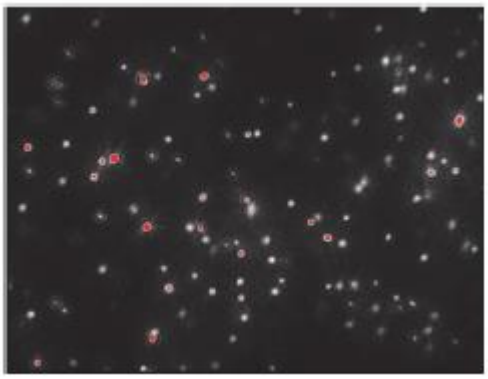
Während der Messung Vibrationen vermeiden!!

Nach der Messung unter **Process Detection Treshold** einstellen, sodass maximal 1-5 blaue Kreuze pro Bild zu sehen sind. Blaue Punkte werden von der Software als Debris erkannt.

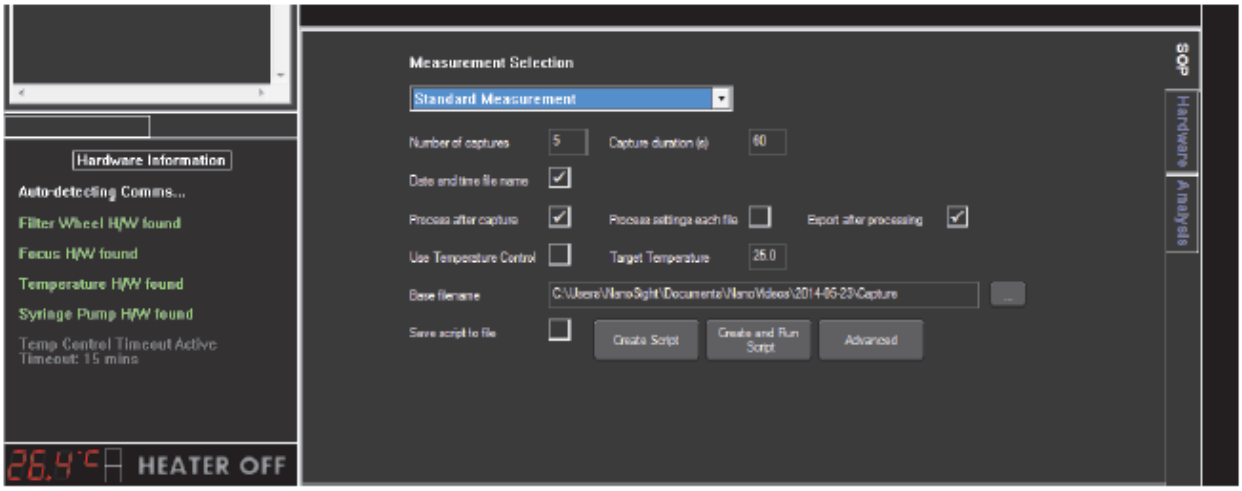
Quick measurement:
30 sec
25°C
Create and Run Script
Set report details anklicken (enthält die Probendetails)
Settings configuration: Settings ok
Process
Detection treshold 5 (fix)

In Excel datei nachschauen, ob Messung ok
Wenn noise level high: blaue +
Particle data
Experiment summary





Standard measurement:



Number of captures (Anzahl der Videos) festlegen: 5 (=5min)

Capture duration (Dauer des Videos) einstellen : 60 (=60sec)

Optional: Use Temperature Control + Target Temperature einstellen

Unter Advanced kontinuierlichen Fluss der Spritzenpumpe einstellen:
Bei O-Ring Top-Plate: recommended flow speed 50-80
Bei Flow-cell Top-Plate: 20-50

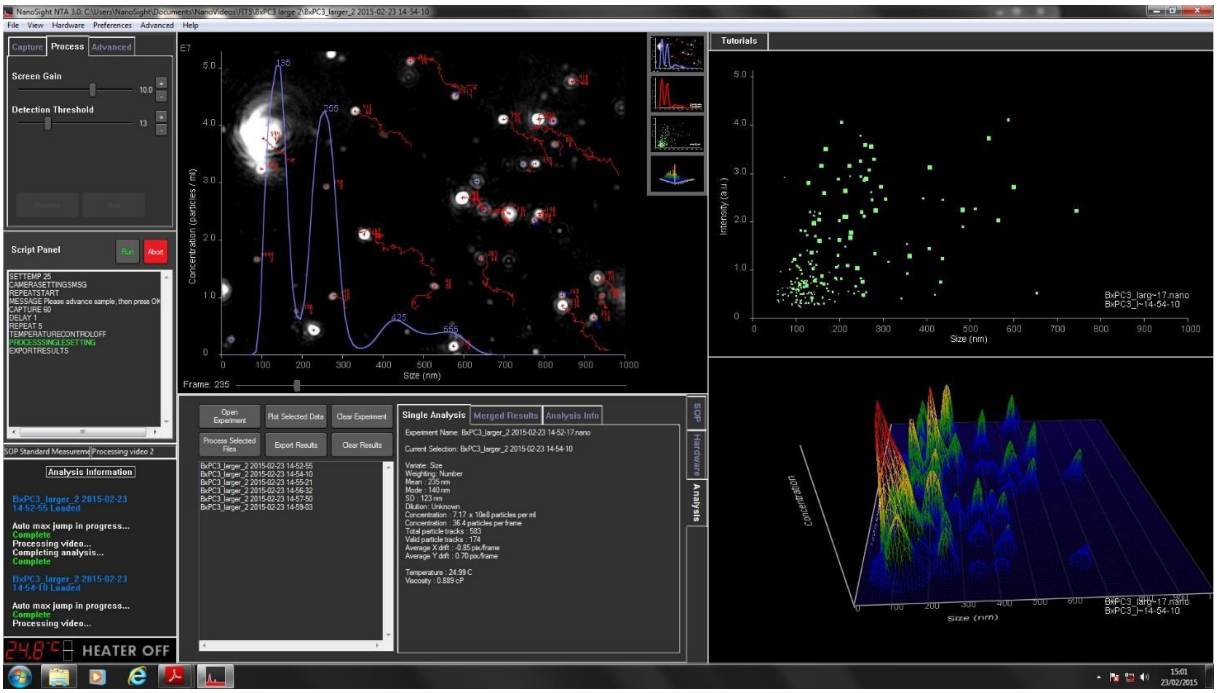
Unter Advanced Viskosität und Verdünnung der Probe eingeben

Unter Base Filename Speicherort und Name des Experiments angeben
Base file name doppelklicken
D:/Nanosight/20161207 (Datum)/F13 1_160 (Probenname und Verdünnung)/
File auswählen, File benennen und save

Advanced Capture settings
Syringe Pump Flow rate anklicken: 50

Create Script:
Script panel (rausziehen nach rechts zum Ändern)
Camera settings
Syringe load 50
Delay 1
Capture 60
Delay 1
Repeat 4
Processing setting
Export results
Script panel mit Kreuz wieder zurückschieben

Auf Create and Run Script klicken, Messung beginnt. Vibrationen vermeiden!



Nach der Messung:

Settings ok anklicken
Script complete ok anklicken
Export results (Daten-Export von Experiment-Report, 10 Sekunden-Video, Experimentzusammenfassung etc.)

Nach der Messung Top-Plate reinigen.

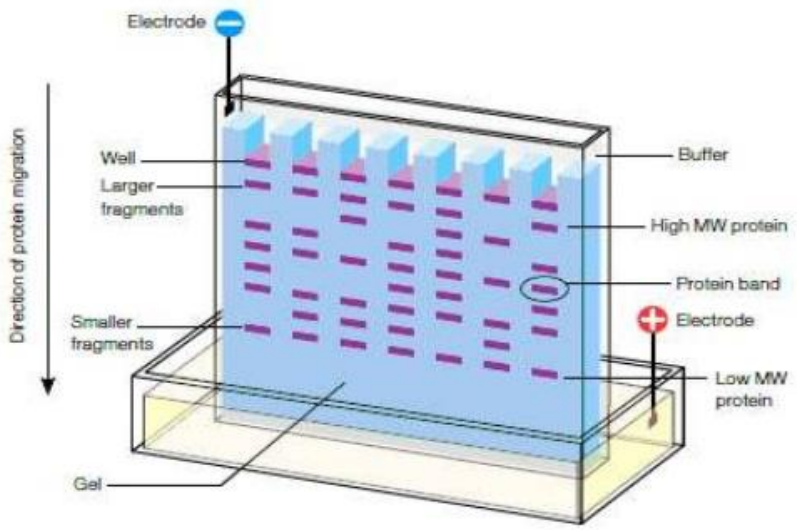
SOP Western Blot

1. Principle & Background

With the western blot technique, you are able to identify specific proteins from a complex mixture of proteins extracted from cells or tissue. The technique uses separation by molecular size (molecular weight) by gel electrophoresis, transfer to a solid support (membrane), and marking target protein using a specific primary and secondary antibody to visualize.

Antibodies bind to specific sequences of amino acids, known as the epitope. Because amino acid sequences are different from protein to protein, antibodies can recognize specific proteins among a group of many. Therefore, a single protein can be identified in a cell lysate that contains thousands of different proteins

First the sample is applied to gel electrophoresis for protein separation as proteins move through a SDS- polyacrylamide gel (SDS PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis) toward the anode with the smaller protein migrating faster and bigger proteins running slower.



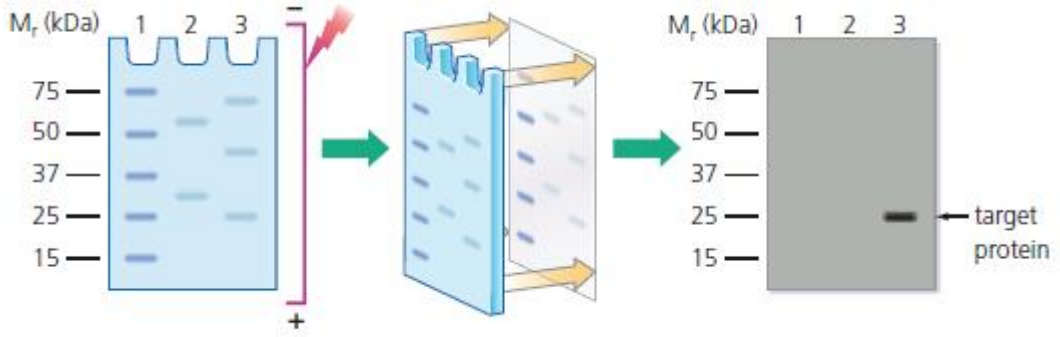
Then the proteins are immobilized on a membrane (nitrocellulose. or PVDF) following electrophoretic transfer from the gel.
Non-protein binding areas on the membrane are blocked in blocking buffer (5% skimmed milk solution) to prevent non-specific binding of antibodies.
The membrane is then incubated with a primary antibody that specifically binds to the protein of interest.
Unbound antibodies are removed by washing and a secondary antibody conjugated to HRP (horseradish peroxidase enzyme) and host specific for the first antibody is applied. For example, if the first antibody was raised in mouse, the second antibody might be termed "goat anti-mouse immunoglobulin".
The most commonly used method for detection is chemiluminescence, based on these secondary antibodies conjugated with the horseradish peroxidase enzyme. After the addition of a peroxide-based reagent, the enzyme catalyses the oxidation of luminol resulting in the emission of light. The light signal can be captured using a charge-coupled device (CCD) camera-based imager

The identity of the bands is confirmed by comparison to molecular weight markers (for size) and a positive control (size and signal).

The detected signal from the protein-antibody-antibody complex is proportional to the amount of protein on the membrane.

Imaging software is then used to compare/calculate the signal intensity generated by the detected

bands.



The Western blot workflow

2. Required Materials

	Name	Supplier	Order number
Devices	Power supply	Amersham	
	Bio-Rad Mini Protean Tetra Cell System	Bio-Rad	???
	Bio-Rad Trans Blot Turbo Transfer System	Bio-Rad	???
Buffers	10 x TBE buffer	Apotheke	
	10 x TGS buffer (1l)	Bio-Rad	161-0732
	Distilled and filtered water	sink	
	Tween 20	Sigma-Aldrich (Merck)	P2287-500ml
	2x Laemmli Sample Buffer	Sigma-Aldrich (Merck)	S3401-10VL
	4x Laemmli Sample Buffer	Biorad	161-0747
Ponceau staining	Ponceau S solution	Sigma-Aldrich (Merck)	P7170-1L
Protein Standards	PageRuler Prestained Protein Marker	Thermo Fisher Scientific	26616
	PageRuler Prestained Plus Protein Marker	Thermo Fisher Scientific	26619
	HiMark Prestained Protein Standard	Thermo Fisher Scientific	LC5699
Blocking reagent	Blotting-Grade Blocker	Bio-Rad	1706404
	Bovine Serum Albumin	Sigma-Aldrich (Merck)	A2153-500g
Bio-Rad Mini Protean precast TGX Gels	10% Mini-PROTEAN® TGX 15-well	Bio-Rad	456-1036
	4-15% Mini-PROTEAN® TGX 15-well	Bio-Rad	456-1086
	4-20% Mini-PROTEAN® TGX..15-well	Bio-Rad	456-1096
	4–20% Mini-PROTEAN® TGX™ Precast Protein Gels, 10-well, 50 µl	Bio-Rad	456-1094
	4–20% Mini-PROTEAN® TGX™ Precast Protein Gels, 12-well, 20 µl	Bio-Rad	456-1095
Bio-Rad Trans Blot Turbo Transfer Pack	Trans-Blot® Turbo™ Mini Nitrocellulose	Bio-Rad	170-4158
	Trans-Blot® Turbo™ Mini PVDF	Bio-Rad	170-4156
Secondary antibodies	Goat-anti mouse	Bio-Rad	170-6516
	Goat-anti rabbit	Bio-Rad	170-6515
	Anti rabbit	R&D	HAF008
	Anti sheep	R&D	HAF016
Substrates	Super Signal West Pico	Thermo Fisher Scientific	34080
	Super Signal West Femto	Thermo Fisher Scientific	34096

3. Reagent preparation

1x TBE Buffer + 0,1 % Tween (TBE-T)

Dilute 100 ml of 10x TBE (Tris Borate EDTA) buffer with 900 ml of Aqua dest. and add 1 mL Tween 20, mix well.

1x TGS Buffer

Dilute 100 ml of 10x TGS (Tris Glycin SDS) Buffer with 900 ml Aqua dest, mix well.

5 % milk blocking buffer

Dissolve 5 g of milk powder (Blotting-Grade Blocker) in 100 ml TBE-T and mix for about 30 min. on a magnet stirrer.

1 % milk blocking buffer

1:5 dilution of 5 % milk with TBE-T (10 ml 5 % milk + 40 ml TBE-T) or
1 g milk powder (Blotting-Grade Blocker) + 100 ml TBE-T

4. Sample preparation

- Prepare proteinlysate samples diluted 1:2 in 2x Laemmli Buffer (stored at -20°C in aliquots) to reach 1x concentration, yielding 8-15 µg of protein per well
- For low concentrated samples you can also use 4x Laemmli Buffer (stored at RT, add 100µl ß-Mercaptoethanol to 900µl 4x Laemmli, dilute sample 1:4 to reach 1x concentration)
- For sample denaturing boil samples for 5 minutes at 95°C in the Eppendorf Thermomixer
- Centrifuge for 3000 rpm, 5min and vortex
- Keep the samples on ice until performing electrophoresis

5. Protein separation by gel electrophoresis

5.1 Selection of appropriate gel

Gel percentage selection depends on the size of the protein of interest. A **4-20% gradient gel** separates proteins of all sizes very well.

Do not run more than one specific gel type in the same apparatus at the same time. Different gel percentages and formulations have different conductivities and different run times.

Protein size (kDa)	Gel percentage (%)
4-40	20
12-45	15
10-70	12.5
15-100	10
25-200	8

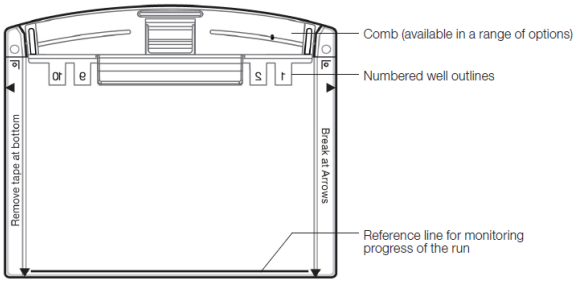
Gel Percentage	Optimum Separation Range	Gel Percentage	Optimum Separation Range
7.5%	40–200 kD	4–15%	20–250 kD
10%	30–150 kD	4–20%	10–200 kD
12%	20–120 kD	Any kD™	10–100 kD

Rule of thumb
The smaller the size of the protein of interest, the higher the percentage of mono/bis is needed.
The bigger the size of the protein of interest, the lower the percentage of mono/bis is needed.

Bio-Rad Mini-PROTEAN precast gels are composed of polyacrylamide with a bisacrylamide cross-linker, and they are available in a range of formulations and in a selection of single percentages and gradients.

Comb Configurations

Comb Type	Well Volume
10-well	50 µl
10-well	30 µl
12-well	20 µl
15-well	15 µl

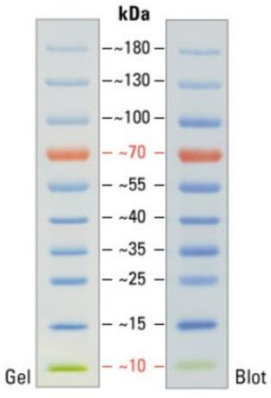
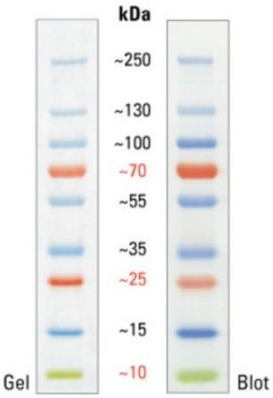
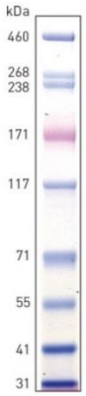


5.2 Positive control

A positive control lysate is used to demonstrate that the protocol is efficient and correct and that the antibody recognizes the target protein, which may not be present in the experimental samples. You will also need this internal positive control for normalization when you quantify your protein bands. Make sure that you have 1 or 2 positive controls on your gel. Use tissue and/or cell protein lysates.

5.3 Molecular weight marker

A range of molecular weight markers will enable the size determination of the protein and allows monitoring of the progress of an electrophoretic run.
Choose the right marker according to the size of your protein of interest:

PageRuler Prestained Protein Marker (Thermo Fisher, #26616)	**PageRuler Prestained Plus Protein Marker (Thermo Fisher, #26619)**	**HiMark Prestained Protein Standard (Thermo Fisher, #LC5699)**
		
Range: 10-180 kDa	Range: 10-250 kDa	Range: 31-460 kDa

5.4 How to use the Mini-PROTEAN Tetra cell

When running 1–2 gels: Use the electrode assembly (with banana plugs), not the companion running module (without banana plugs). Do not place the companion running module in the tank. Doing so generates excessive heat and degrades the quality of the electrophoretic separation.

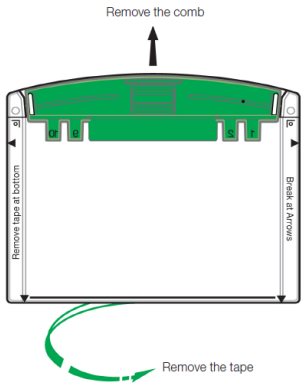
When running 3–4 gels, use both the electrode assembly and companion running module. When using voltages >200 V, fill the outer buffer chamber to the 4 gel (800 ml) mark.

Mini-PROTEAN® Tetra Companion Running Module without banana plugs (Bio-Rad, #1658038)

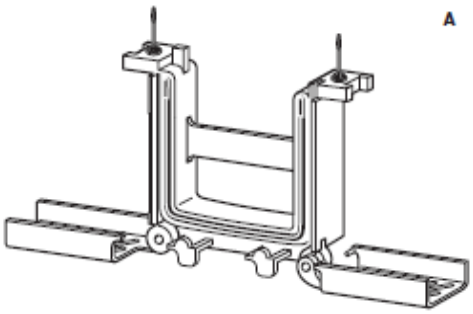
Mini-PROTEAN® Tetra Electrode Assembly with banana plugs (Bio-Rad, #1658037)

5.5 Setting up and Running Mini-PROTEAN Gels in the Mini-PROTEAN Tetra Cell

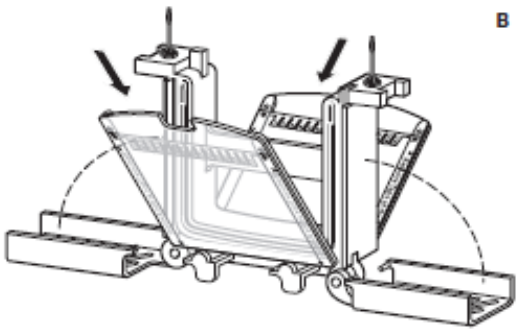
- Remove the Bio-Rad precast Mini Protean TGX Gel gels from the storage pouch and prepare them for assembly
- Rinse with AD to remove the storage buffer
- **Remove the green tape: Pull gently to remove the green tape from the bottom of the cassette. (VERY IMPORTANT!!!)**
- Remove the comb: Position thumb on the indentation (middle of comb) and remove the comb by pulling upward in one smooth motion – take care of not destroying the wells!
- Sometimes air bubbles get formed between the gel and plastic plate – press them out! This might cause bad running performance of single samples

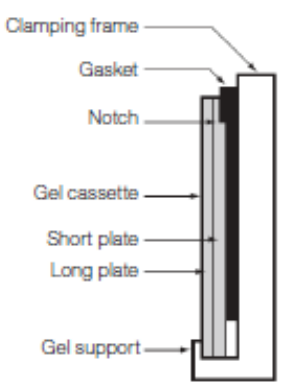


- Set the electrode assembly to the open position on a clean, flat surface (A).

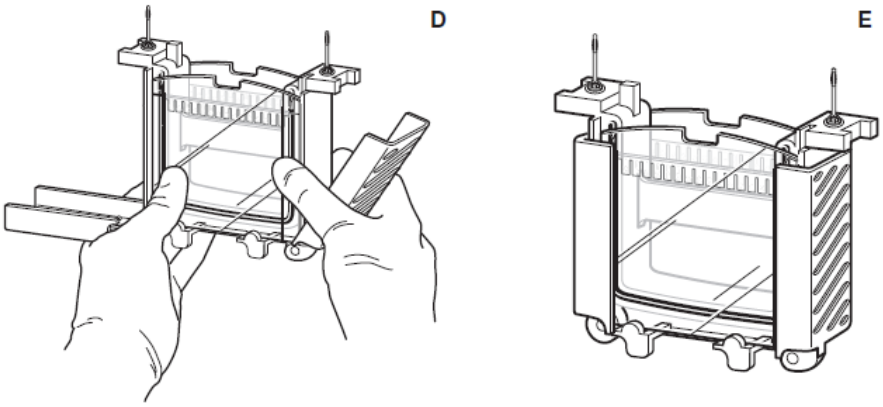


- Place the first cassette with the short plate facing inward and so the gel rests at a 30° angle away from the center of the electrode assembly. (B)
- Place the second gel or buffer dam on the other side of the electrode assembly (B).
  Two cassettes are required to create a functioning assembly; when using 1 or 3 gels, use the buffer dam (included with the cell) to complete the assembly.
- Gently push both gels toward each other, making sure that they rest firmly and squarely against the green gasket that is built into the electrode assembly (C).

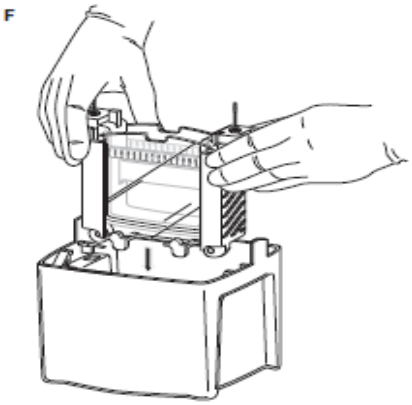




- While gently squeezing the gel cassettes (or cassette and buffer dam) against the green gaskets (maintaining constant pressure and with both gels in place), slide the green arms of the clamping frame one at a time over the gels, locking them into place (D,E).
- The wing clamps of the electrode assembly lift each gel cassette up against the notch in the green gasket, forming a seal. Check again that the short plates sit just below the notch at the top of the green gasket (C).
- If running more than 2 gels, repeat steps with the companion running module.



- Place the electrophoresis module into the tank (F) and fill the buffer chambers with 1x running buffer (1x TGS buffer):
  200 ml in the inner buffer chamber, 550 ml (1–2 gels) or 800 ml (3–4 gels, or >200 V) in the outer buffer chamber.
- Check for leakage!!!



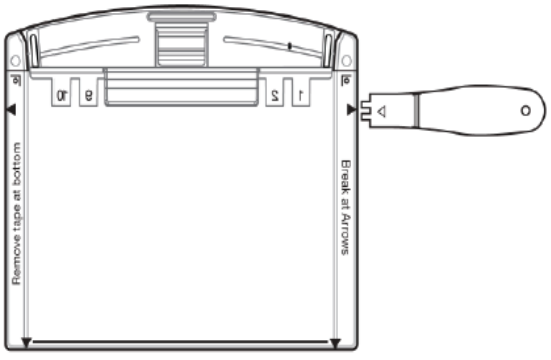
- Rinse the sample wells with running buffer (1x TGS buffer) using a syringe or pipet. Straighten the sides of the wells, if necessary.

5.6 Loading the samples, positive controls and protein markers

- Load equal amounts of protein (8 – 15 µg) into the wells of a mini format SDS-PAGE gel, along with the molecular weight markers (5 µl).
- After sample loading run gel at **110 V, 400 mA for around 1h 10 min**.
    - For separation of larger proteins > 200 kD it's better to run 90V, 10mA, ~3h
- Check the gel every few minutes to make sure the lanes are running evenly.
- Stop the run when the dye front reaches the reference line imprinted on the bottoms of the cassettes.

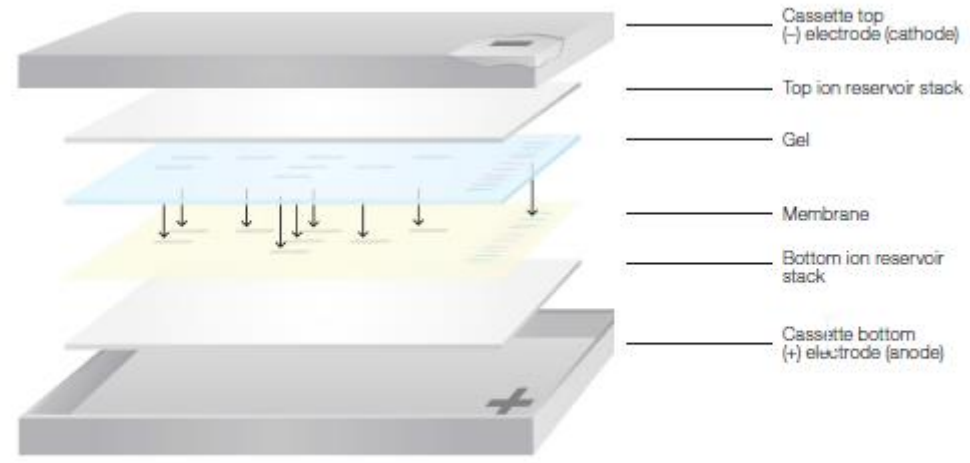
5.7 Removing the Gel

- After electrophoresis is complete, turn off the power supply and disconnect the electrical leads.
- Remove the lid from the tank and remove the gels from the cell. Pour off and discard the running buffer.
- To open the cassette, align the arrow on the opening lever with the arrows marked on the cassette and insert the lever between the cassette plates at indicated locations. Apply downward pressure to break each seal.
- Pull the two plates apart from the top of the cassette, and gently remove the gel.



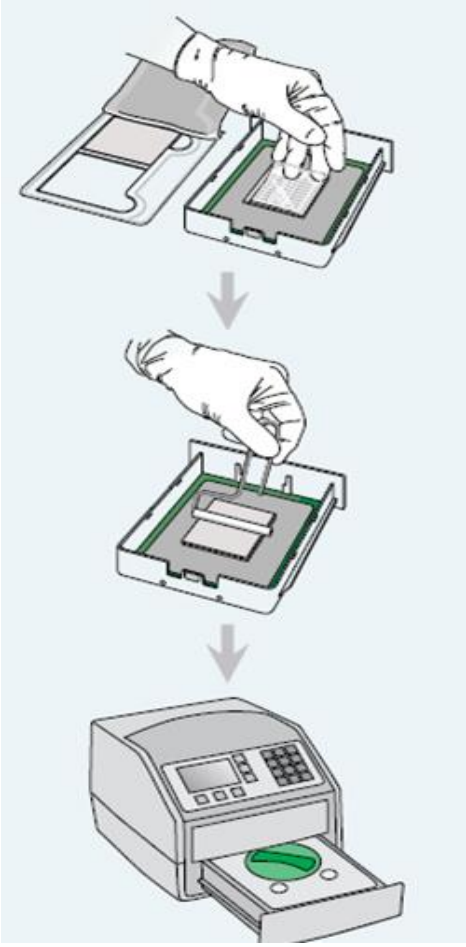
6. Blotting - Transfer of the protein from the gel to a membrane using the Trans-Blot® Turbo™ System

- Open the transfer pack and assemble the components on the cassette in the order shown below:



Assembly of the gel blot sandwich with the Trans-Blot Turbo system.

- To make sure no air bubbles are trapped in the sandwich, use the roller to remove any air trapped between the layers.
- Once the membrane is on the gel, do not move or remove it any more, as you might already have a protein smear on the membrane!
- The blot should be on the cathode and the gel on the anode, therefore place gel on top of the membrane.
- Place the sandwich in the cassette.
- Place the lid on the cassette and lock the lid in place by turning the knob clockwise, using the symbols on the lid as a guide.
- Slide the cassette into the appropriate bay of the Trans-Blot Turbo cell. Each cassette and bay can hold up to two mini gels or one midi gel.



- Start the transfer: press LIST and BIO-RAD to select a Bio-Rad optimized protocol or a user-defined protocol. Press NEW to create and run a new protocol.
- Select kind and number of gels
- Select one of the predefined programs depending on the molecular weight of the protein of interest, according to the table below.
- Press A:RUN or B:RUN to begin the transfer.

Table 11.2. Trans-Blot Turbo transfer protocols.

Protocol Name	MW, kD	Time, Min	1 Mini Gel	2 Mini Gels or 1 Midi Gel
STANDARD SD	Any	30	Up to 1.0 A, 25 V constant	Up to 1.0 A, 25 V constant
1.5 MM GEL	Any	10	2.5 A constant, up to 25 V	1.3 A constant, up to 25 V
HIGH MW	>150	10	2.5 A constant, up to 25 V	1.3 A constant, up to 25 V
LOW MW	<30	5	2.5 A constant, up to 25 V	1.3 A constant, up to 25 V
MIXED MW	5–150	7	2.5 A constant, up to 25 V	1.3 A constant, up to 25 V
1 Mini TGX	5–150	3	2.5 A constant, up to 25 V	N/A

- When the transfer is completed, RUN COMPLETE appears on the screen.

- Remove the cassette from the instrument and unlock the lid. **Caution -** the cassette may be warm! Remove the membrane from the transfer sandwich and discard the remaining transfer pack materials.
- **Always clean the cassette and Trans Blot Turbo Cell with Aqua dest. and Bacillol AF tissues. In case of persistent contaminations use 70 % Ethanol and work with forceps to remove impurities!**

7. Ponceau S Staining – Visualization of proteins

Checking protein transfer quality is useful for determination of a uniform and even migration of proteins.

- Stain the blot with Ponceau S for 5 minutes on a shaker
- Destain the blot by washing with distilled water. Proteinbands should stay visible.
- Put stained membrane on a sheet protector and take a picture.
- Briefly wash the membrane with 1 x TBE buffer + 0.1%Tween to remove Ponceau S completely.

8. Blocking the membrane

Blocking the membrane prevents non-specific background binding of the primary and/or secondary antibodies to the membrane, as it has a high capacity at binding proteins and therefore antibodies.

- Block membranes with 5 % milk blocking solution for 1 h at room temperature on a horizontal shaker with gentle agitation.
- **Do not wash after blocking!!!** Immediately apply the primary antibody solution.

Note! Two blocking solutions are traditionally used: non-fat milk or BSA. Milk is cheaper but is not recommended for studies of **phosphoproteins**. Milk contains casein which is a phosphoprotein and therefore causes high background.
For blocking in BSA, a 2% BSA-TBE solution containing 0.1% Tween is recommended.

9. Primary and Secondary Antibody incubation

The antibody should be tested in the dilutions recommended in the datasheet (check literature too). Do a test run with 2 - 3 dilutions and optimize the protocol depending on the results. Too high antibody concentrations will result in non-specific bands.

- Add the primary antibody solution against the target protein diluted in the 1 % milk blocking solution.
- Seal the membrane covered with the primary antibody solution in a small trash bag.
- Incubate overnight in the freezer at 4°C with gentle agitation on a vertical shaker.

Incubation Time

The time can vary between a few hours and overnight (rarely more than 18 hours), and is dependent on the binding affinity of the antibody for the protein and the abundance of protein. We recommend a more diluted antibody for a prolonged incubation to ensure specific binding.

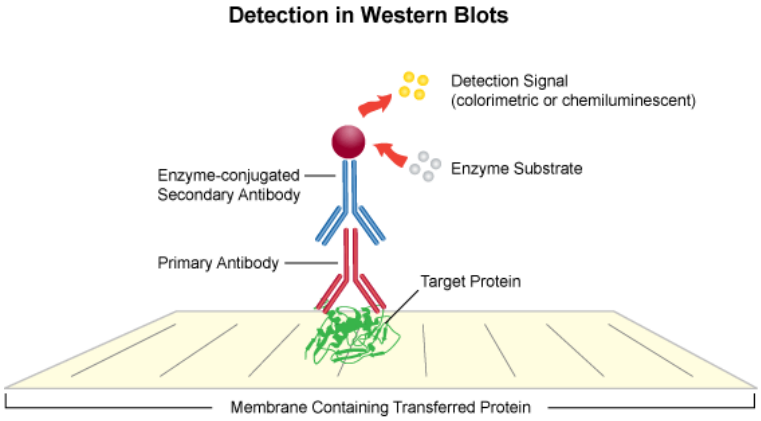
Incubation Temperature

Preferably cold. If incubating in blocking buffer, it is imperative to incubate at 4°C or contamination will incur and thus destruction of the protein overnight (especially phospho groups).

Agitation

Agitation of the antibody is recommended to enable adequate homogenous covering of the membrane and prevent uneven binding.

- The next day wash the blot extensively in wash buffer (1 x TBE buffer + 0.1%Tween) for 1 hour, changing the buffer every 10 minutes with gentle agitation on a horizontal shaker.
- Add appropriate secondary HRP-conjugated secondary antibody diluted in 1 % milk blocking solution and incubate for 1 hour at room temperature with gentle agitation on a vertical shaker.
- Wash the blot again in wash buffer (1 x TBE buffer + 0.1%Tween) for 1 hour, changing the buffer almost all 10 minutes with gentle agitation on a horizontal shaker.



10. Detection and imaging

Use Bio-Rad SuperSignal West Pico (enables picogram detection) or SuperSignal West Femto (enables femtogram detection) as a chemiluminescent substrate for developing.

- Prepare Working Solution by mixing equal volumes (1:2) of the Stable Peroxide Solution and the Luminol/Enhancer Solution. Use 0.1mL Working Solution per cm^2 of membrane (approx. 3 ml per membrane). The Working Solution is stable for 24 hours at room temperature.
- Incubate the blot with Working Solution for 5 min at room temperature on a sheet protector
- Remove Working Solution from the blot and drain excess reagent
- Place the blot protein-side-up in a clear transparent foil and remove air bubbles
- Take images using the Vilber imaging system (see “SOP_Fusion FX Western Blot Imaging”)
- In case your signal is to intense and causes bleaching of the protein band, dilute SuperSignal West Femto or Pico 1:3 in 1x TBE buffer

11. Stripping of membranes with Restore™ Western Blot Stripping Buffer

Material:

- 21059 Restore Western Blot Stripping Buffer, 500mL
- Western blot, previously blocked, probed and detected with chemiluminescent substrate
- 1x TBE+ 0.01% Tween
- Primary and secondary antibodies for both first and second Western blotting experiments

Procedure:

- Blots may be stored in 1 x TBE + 0.01 % Tween at 4°C until the stripping.
- Place the blot in Restore Western Blot Stripping Buffer and incubate for 20 minutes at room temperature
- Use a sufficient volume to ensure that the blot is completely covered (~20mL)
- Remove the blot from the Restore Western Blot Stripping Buffer and wash in 1 x TBE + 0.01% Tween) at room temperature for about 45 minutes
- Used Western Blot Stripping Buffer is collected in a glass bottle and will be reused
- Block membrane again with 5% milk for 1h at RT or ON at +4°C
- Blot is now ready for next antibody incubation

Notes:

- Optimization of both incubation time and temperature is essential for best results. In general, high-affinity antibodies will require at least 15 minutes of stripping and might require incubating at 37°C.
- Blot can be stripped and reprobed several times but might require longer exposure times or a more sensitive chemiluminescent substrate.
- Subsequent reprobings might result in decreased signal.
- Reblocking a membrane is usually not necessary after stripping, but might be required in some applications