Questions & Answers

Rapid, Accurate Exosome Purification and Sizing: High Performance Exosome Purification and Characterization via automated Density Gradient, Ultracentrifugation and Dynamic Light Scattering

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1.- Which mechanism does exosomes follow to enter into a cell?

Exosomes enter the cell through both endocytosis as well as the membrane of the exosome fusing with the membrane of the acceptor cell.

2.- Is there any signaling or sequence that allows mRNA and miRNA packing inside exosomes?

There is some research done showing preference for 3'UTR mRNA transcripts. But there is still more work to be done before the complete mechanism is understood. (Exosomes secreted by human cells transport largely mRNA fragments that are enriched in the 3'-untranslated regions. Biology Direct. 2013; 8: 12. - Arsen O Batagov and Igor V Kurochkin)

3.- Are exosomes produced by neurons?

Yes, research in the past 3 years has shown that neurons are released by exosomes (Lachenal, et. al. Mol. & Cell. Neuroscience, 2011)

4.- What determines what is or is not included in exosomes from the host cell?

There is a lot of research being done on the pathways for protein packaging in exosomes. Not all of it is conclusive yet. A good review is -Urbanelli, L.; Magini, A.; Buratta, S.; Brozzi, A.; Sagini, K.; Polchi, A.; Tancini, B.; Emiliani, C. Signaling Pathways in Exosomes Biogenesis, Secretion and Fate. Genes 2013, 4, 152-170.

5.- Can they play a role in neurone communication and metabolism?

Yes, exosomes have been shown to play a role in neuron-glia communication (Frühbeis et. al PLOS Bio, 2012) and they play a major role in regulating metabolism and have been examined as markers of metabolic diseases such as diabetes.
6. What are the volumes of cell culture supernatants to purify exosomes by ultracentrifugation?

We used about 40 mls supernatant for the application note. However there are papers showing 3-4 mls of samples used.

7. How difficult is to analyze mass spc patterns to differentiate mRNA sequences in exosomes?

Typically, researchers use mass spectrometry to analyze the protein and peptide sequences contained in exosomes, as well as the translated protein products from the mRNA contained within exosomes. As far as I am aware, mass spectrometry to differentiate mRNA sequences is not used; Next Generation Sequencing is more common at this time.

8. Why do several rounds of 10,000xg centrifugations to remove unwanted particles, rather than just a single 10,000xg spin?

To clarify, in the exosome centrifugation purification process, there is only a single round of 10,000xg centrifugation to remove unwanted microvesicles and cellular debris from 200 nm to 1000 nm in diameter. My apologies for any confusion.

9. What is the difference between exosomes and microvesicles?

Exosomes are smaller in diameter than microvesicles (30-120 nm vs. 100-1000 nm). They have different protein markers and play different roles in cellular communication and genetic exchange. For more information, please consult the following reference (Ling, et. al., Frontiers in Immunology, 2011; Duijvesz e. al., European Urology, 2011)

10. What volume of serum or plasma would be required to realistically identify sufficient exosomes to work with. No doubt depends on the concentration, but more or less?

This is a difficult question to answer without more details. The research being done with the exosomes and purification technique will all play a role in the necessary volume.

11. What is the yield in general after step 4?

The exact yield of the exosomes after step 4 is unknown.

12. What are your thoughts on using filter centrifugation as a method to remove those protein contaminations? (I have used 100k filter membranes)

Filter centrifugation will remove some protein contamination. However, some portion of the proteins will adhere to the membrane of the exosome, preventing quick filtration; long time periods of density gradient ultracentrifugation helps avoid this type of protein contamination.

13. What is the volume for ultracentrifugation?

The volume for each step of ultracentrifugation (Step 2, 3 and 4) are all different. For the density gradient ultracentrifugation (step 4), the volume of the centrifuge tubes are ~ 12 ml.

14. Do you have information about Fc receptors on exosomes?

We did not do any membrane protein studies on the exosomes.
15.- What is the difference (functional too) between an exosome and a microvesicle?

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16.- Will size-exclusion gel chromatography work?

Size exclusion chromatography, coupled with a dynamic light scattering instrument, could possibly isolate exosomes. However, the low concentration of exosomes in most bodily fluids mean that a concentration step is necessary, usually performed with ultracentrifugation at speeds high enough to pellet exosomes.

17.- You mentioned that it is important to remove microvesicles from your purified exosome population, because microvesicles have a different functional role compared to exosomes. Could you expand on this?

Exosomes are smaller in diameter than microvesicles (30-120 nm vs. 100-1000 nm). They have different protein markers and play different roles in cellular communication and genetic exchange. For more information, please consult the following reference (Ling, et. al., Frontiers in Immunology, 2011; Duijvesz e. al., European Urology, 2011). Microvesicles contaminating an exosome sample could influence the results of many experiments, including protein assays, RNA assays, etc.

18.- What is the ball park price of the Delsamax Pro and can it also be used for large nanoparticles?

The DelsaMax PRO can be used to analyze large nanoparticles by size and zeta potential up to several microns in size.

19.- Hi! How much is a DelsaMax Core approximately? Thank you for answering and thanks for the nice webinar!

Thank you. Please see www.delsamax.com

20.- Have you compared commercially available exosome purification reagents with ultracentrifugation method?

At this time, we at Beckman Coulter Life Sciences have not compared commercially available exosomes purification kits with ultracentrifugation. That is going to be an area of future research focus.

21.- Can you provide the SAMI protocol for making gradients on the FX?

We did not use a SAMI protocol for this app note. However if the customer has SAMI and wants to this can be customised on it.

22.- Thank you very much. I am more interested in membranes of exosomes. Is it known how completely the membrane of exosomes resembles the one of the original cell with respect of its lipid and protein composition (e.g. content of tumor antigens)?

The protein and lipid composition will be unique and different from the membrane of its cell of origin. There is a great deal of overlap of proteins, but it will vary on a cell by cell basis. Certain proteins are more highly concentrated in the membranes of all exosomes compared with cellular membranes, including proteins in the tetraspannin family.

23.- Which other cells do you use for exosomes isolation?

Exosomes have been isolated from many cells, including B cells, T cells, Dendritic cells, mast cells, and many others. Please see the following review article for a more comprehensive list: (Théry, Clotilde, Laurence Zitvogel, and Sebastian Amigorena. "Exosomes: composition, biogenesis and function." Nature Reviews Immunology 2.8 (2002): 569-579.)
24.- How could I identify the cells source of exosomes identified in plasma or some biological fluid?

Protein and membrane markers can be used as a way to help identify the cellular source of the exosomes.

25.- Can you scale up for GMP production?

Depending on the application of the exosomes, scale-up to GMP production should be theoretically possible, though it has not yet been put into practice, as far as I am aware.

26.- Will CD4 lymphocytes release exosomes as part of their role in the immune response

Exosomes actually play a role in activating CD4 cells; dendritic cells will release exosomes that activate CD4 cells (Théry, Clotilde, et al. "Indirect activation of naive CD4+ T cells by dendritic cell-derived exosomes." Nature immunology 3.12 (2002): 1156-1162.)

27.- Is the surface charge on exosomes cell dependent?

Surface charge on exosomes will be solvent dependent and is dependent on the protein and lipid composition of the membrane. Since the protein content does show cell dependence, there may be a shift in zeta potential/surface charge for exosomes based on cell of origin. However, to my knowledge there has been no comprehensive studies on this to date.

28.- Can we re-construct any type of exosomes in lab as cargo to move mRNA, etc?

Cell lines can be grown up in a lab and exosomes containing mRNA can be harvested; however, construction of exosomes from scratch in a lab would be very difficult.

29.- I am working with synthetic peptide. I want to measure the molecular weight of the peptide. Its 28 aa sequence. How can i use dynamic light scattering for the same?

Yes, using the DelsaMax CORE, which combines dynamic light scattering and static light scattering, you can get very accurate molecular weight for your peptide (the molecular weight range goes down to 300 Da). For more information, visit www.delsamax.com

30.- Could exosome preps for virus contaminated?

Exosome prep could have some viral contamination if the exosomes are harvested from in vivo source and the viral vector has a similar size and density to that of exosomes. However, many virus and viral vectors are of higher density than exosome, so the Density Gradient Ultracentrifugation step of purification should eliminate most contamination.

31.- How does the machine calculate the mass% out of the scattering intensity?

The DelsaMax CORE calculates %mass from scattering intensity% using Mie theory.

32.- In your Jurkat culture, what kind of culture medium did you use? Was it a low-serum or serum-free medium?

The Jurkat cells were cultured in RPMI-10%FBS. The FBS was ultracentrifuged to remove any bovine exosomes before adding it to the culture media.

33.- how much of a problem is aggregation post-purification

We saw no problem with exosome aggregation post-purification in 1x Phosphate Buffer Saline. Aggregation would have appear as larger diameter peaks in the dynamic light scattering analysis. Other research does not indicate much aggregation of exosomes after purification based on electron microscopy.

34.- Do you know if there is a good method to purify exosomal-like particles which reside inside a cell? The cells have to be cracked mechanically, of course, but what purification should follow?
Technically a differential centrifugation type of method followed by a DGU could be used for something like this. Although the exact protocol would depend on variables like the size/composition/density and location of the particles. As well as any specific precautions needed to preserve the integrity of the particle during lysis and purification.

35.- Is there a technique using HPLC or another chromatography techniques to optimize exosomes purification?

Chromatography could be used for exosome purification; however, this will dilute the exosome concentration, which is already very low. A concentration of the exosomes by centrifugation pelleting before and after the HPLC would be needed.

36.- This is more of a comment than a question; I feel there should be a section on the relevance for prognosis/diagnosis of diseases including cancer. The point is, Why would a lab want to invest in research on exosomes?

Numerous publications over the past few years have indicated that exosomes have both pro-tumor and anti-tumor effects. For example, exosomes from tumor cells have been shown to help mediate the immune response to the cancerous cells.

37.- Are there consumables involved with the instrument? What is the cost to run 1 sample?

There are no consumables used to run the DelsaMax CORE or PRO. The only consumable in the process is the centrifuge tubes, which are of negligible cost.

38.- Is there any service to analyze a sample for sizing?

Contract Research Labs have access to dynamic light scattering instruments (as well as electron microscopy, Image tracking analysis, etc.). My recommendation is to contact such a lab to look for exosome sizing service.

39.- Why not use gel filtration for getting rid of contaminants proteins?

Gel filtration will remove some protein contamination. However, some portion of the proteins will adhere to the membrane of the exosome, preventing quick filtration; long time periods of density gradient ultracentrifugation helps avoid this type of protein contamination. Also, gel filtration may cause some loss of exosome sample.

40.- Do you incubate your cells of interest in FCS-free media (or conditioned media) before you start the isolation-procedure? And how long do you incubate the cells in this media?

No, we did not incubate the Jurkat cells in FCS-free media. We ultracentrifuged the FBS (or FCS) to remove any bovine exosomes before adding it into the culture media.

41.- I centrifuge at 16000rpm for 30 min. Is that ok to continue for MP detection?

"It is tough to tell how effective centrifugation when reported in RPM, since it does not correct for the distance from the center of the rotor to the sample. Also, the size and density of the Microparticle will also play a role in g-force and time needed. When working with exosomes, we found that, 100,000 g is needed for at least 60 minutes to ensure that exosomes are pellet out."

42.- How should I clean my centrifugation tubes?

The method you chose to clean/sterilize the centrifuge tubes would depend on your downstream needs. We have a wide variety of centrifuge tubes available. The tubes can be hand washed with mild detergent and some of this tubes can be autoclaved or cold sterilized. Please refer to bulletin IN-175 for chemical compatibility.

43.- What percentage of exosomes remain in solution after pelleting by centrifugation?

We did not attempt to quantify the percentage of exosomes in the supernatant vs. the pellet in our research yet. In the future, we will examine the loss of exosomes at each step.
44.- Do you think that the western blot is also a good method to analyze exosomes in samples?

Western blot has been used and can be used to test the protein content of exosomes.

45.- Can the delsamax be used for general nanoparticle characterization also?

Yes, the DelsaMax can be used for general nanoparticle size, stability, and molecular weight characterization.

46.- Can DLS be combined with phenotyping with bead labeled antibodies?

Dynamic light scattering is probably not the ideal instrument to use for phenotyping; while it could determine the size of the bead-labeled antibodies, DLS is not sensitive enough to reliably detect the subtle size needed for the experiment I believe you are proposing.

47.- Can you see the pellets of exosomes after ultracentrifugation?

Yes, the exosomes appear as a small, clear pellet if they are in high enough concentration.

48.- What is the typical period that cells should be cultured to accumulate exosomes in media for downstream purification?

The typical period for culturing would be cell line dependent. In general though exosome purification should be done from healthy cells with more than 95% viability when the media is harvested.

49.- Is ultrafiltration system comparable to ultracentrifugation in terms of purity and yield of exosomes after purification?

Although we have not yet had a chance to compare the two technologies, one common drawback of ultrafiltration is the co-purification of proteins and other smaller contaminants, as well as loss of exosomes due to getting trapped in pores.