A Beginner's Guide to Exosome Isolation

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For all of you who have never heard of exosomes: You are missing out on a whole new paradigm in cell-to-cell communication. Exosomes are tiny extracellular vesicles that arise from fusion of the plasma membrane with specific endosomal compartments called multivesicular bodies. Most cells types make exosomes, and release them in order to communicate with neighboring cells.

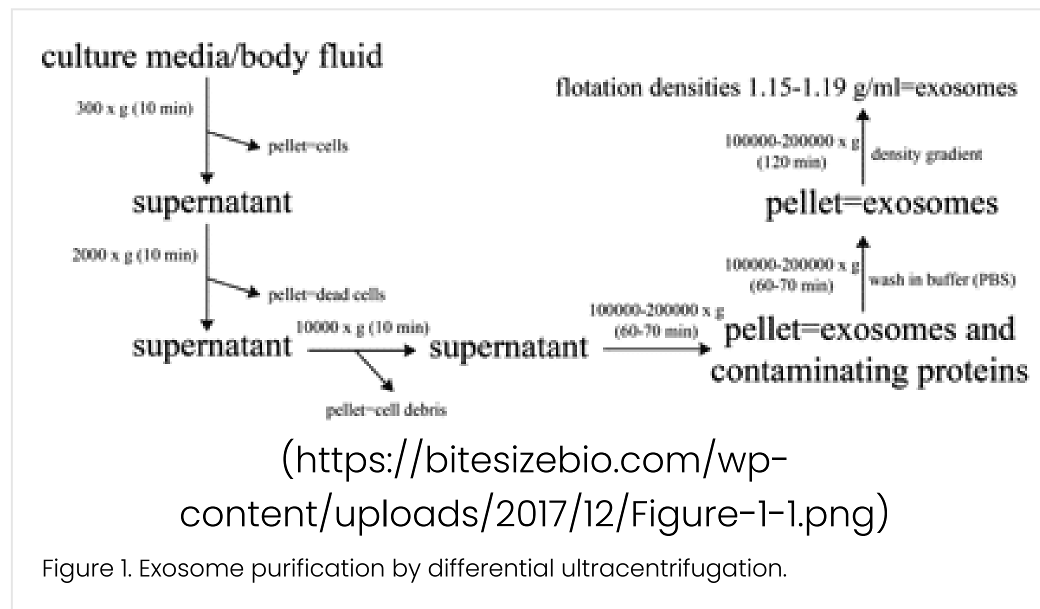
Exosomes contain a plethora of information within DNA, microRNA, mRNA, and proteins, and good exosome isolation techniques are necessary in order to tap into all the information they store. Here, I will introduce you to four of the most common exosome isolation techniques.

Ultracentrifugation-Based Exosome Isolation

You can separate exosomes from other sample components via differential centrifugation. You do this by sequentially centrifuging your samples at increasing speed (Figure 1), in order to remove contaminants such as cells and cell debris. Finally, by ultra centrifuging the resulting supernatant at high speeds, you pellet and purify the exosomes from contaminating soluble molecules such as free proteins and protein complexes.

It is important to wash pelleted exosomes with PBS or fresh growth medium at least once to purify them from free protein remnants that originate from the lower mobility fraction. You must perform all centrifugation steps at 4ºC to maintain proteases, DNases and RNases in an inactive state.

This method is often used in combination with sucrose density gradients or sucrose cushions to further purify exosomes according to their density. For this setup, you would centrifuge the pellet (containing the exosomes) at 100,000–200,000 x g for 120 min in a centrifuge containing a pre-constructed sucrose gradient medium, with decreasing sucrose density from bottom to top. Exosomes should float at sucrose densities ranging from 1.15 to 1.19 g/mL on continuous sucrose gradients.



(https://bitesizebio.com/wp-content/uploads/2017/12/Figure-1-1.png)

Figure 1. Exosome purification by differential ultracentrifugation.

Although combining these two methods to yield highly purified exosome preparations is popular, there are some drawbacks. The procedure is time consuming and labor intensive. It also requires a large amount of starting material. Furthermore, one of the biggest pitfalls of this isolation technique is that you can “over do it.” For example, if you prolong the centrifugation times, the exosomes may disintegrate, or any soluble proteins present could form aggregates and pellet along with the exosomes to contaminate the final sample.

Size-Based Exosome Isolation

These techniques separate exosomes on the basis of their size, and include ultrafiltration and size-exclusion chromatography (SEC). Ultrafiltration is a faster method than ultracentrifugation and doesn’t require special equipment. If you study exosomal RNA and want to isolate it quickly this should be your method of choice, because it leads to greater RNA yields than ultrafiltration and precipitation. Make sure

to wash out the filters after each step to improve exosome recovery. To maximize isolation speed, you could use one of many available kits that remove cell debris and other contaminants on one micro filter and capture exosomes on a second.

Alternatively, for rapid exosome enrichment, you may use nano-filtration concentrators with short periods of centrifugation. Sequential filtration is an effective way to isolate exosomes from cell culture supernatant. You perform this technique by sequentially changing filters with different molecular weight cut-off values. The main drawback of ultrafiltration is that exosomes can sometimes aggregate and clog the filter pores.

In SEC, an in-column stationary porous phase is used to separate macromolecules and particulate matter according to their size. Small molecules are able to pass through the pores, resulting in late elution, while larger components (including exosomes) are excluded from entering the pores, thus resulting in early elution. I find this method to be very efficient in combination with differential centrifugation for obtaining highly purified exosome preparations.

Precipitation-Based Exosome Isolation

You can possibly force less-soluble molecules and exosomes out of solution by tying up water molecules. To this end, you can use volume-excluding polymers, such as polyethylene glycol (PEG) 8000 kDa. A typical protocol begins with incubating samples with PEG at 4ºC overnight. After that, you can easily recover your precious exosomes from the resulting precipitate by either low-speed centrifugation or filtration. Alternatively, if you know the glycan composition of your exosomes, it may be worthwhile using lectin-induced exosome agglutination.

Lectins are proteins that bind specifically to carbohydrates, and induce cell agglutination. We can use lectins to agglutinate exosomes and pellet them by centrifugation with a relatively low G-force. Finally, the easiest but most expensive precipitation-based methods include a range of commercially available reagents and kits.

Immunoaffinity-Based Exosome Capture

Because exosomes are rich in proteins and contain many receptors on their surfaces, the ultimate exosome isolation technique involves capturing them with antibodies. You can achieve this using antibodies specific for some of the most common exosomal protein markers, such as: CD9, CD81, CD63, CD82, Hsp70, Ras-related protein Rab-5b, cytoskeletal protein actin and TSG101.

You can use specific antibodies to these exosomal markers to select desired exosome population (immunoenrichment) or to trap unwanted exosome populations (negative selection or immunodepletion). Because exosomes are very heterogeneous in accordance to their origin, abundance of these markers on different exosomes also varies. So, you may use combination of specific antibodies to capture different types of exosomes from your sample. We do this by immobilizing these antibodies on ELISA plates, magnetic and chromatography beads, or use them in microfluidic devices.

Immunoaffinity techniques have the potential for high specificity, something you should consider if you are characterizing unique exosome populations. However, because some markers are possibly not represented or recognized on all exosomes within your sample, this high selectivity comes with a price. That is, we get lower yields compared to methods that rely on physical properties. Even so, this method produces the purest exosomes.

You Have Your Exosomes, Now What?

So, why would you go through all this trouble to study exosomes? Well, exosomes have gained significant interest in recent years, not only because they are involved in many biological processes, but also because accumulating evidence indicates that they are associated with malignant transformation of cells. Furthermore, their appearance in body fluids such as urine, milk, blood, semen, and plasma warrants further study into their potential as valuable biomarkers for disease and to aid the development of new therapeutics.

If this article intrigued you, and you want to study these phenomenal tiny vesicles, remember that each isolation technique has its advantages and disadvantages. Each technique exploits one or more unique exosomes traits, such as density, shape, size, and surface protein constitution. Also, bear in mind that you are not bound to just one isolation technique. You might find that using several techniques in combination yields the more pure exosomes from your sample type. Let the hunt begin!

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Rosie on March 13, 2020 at 9:43 am

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