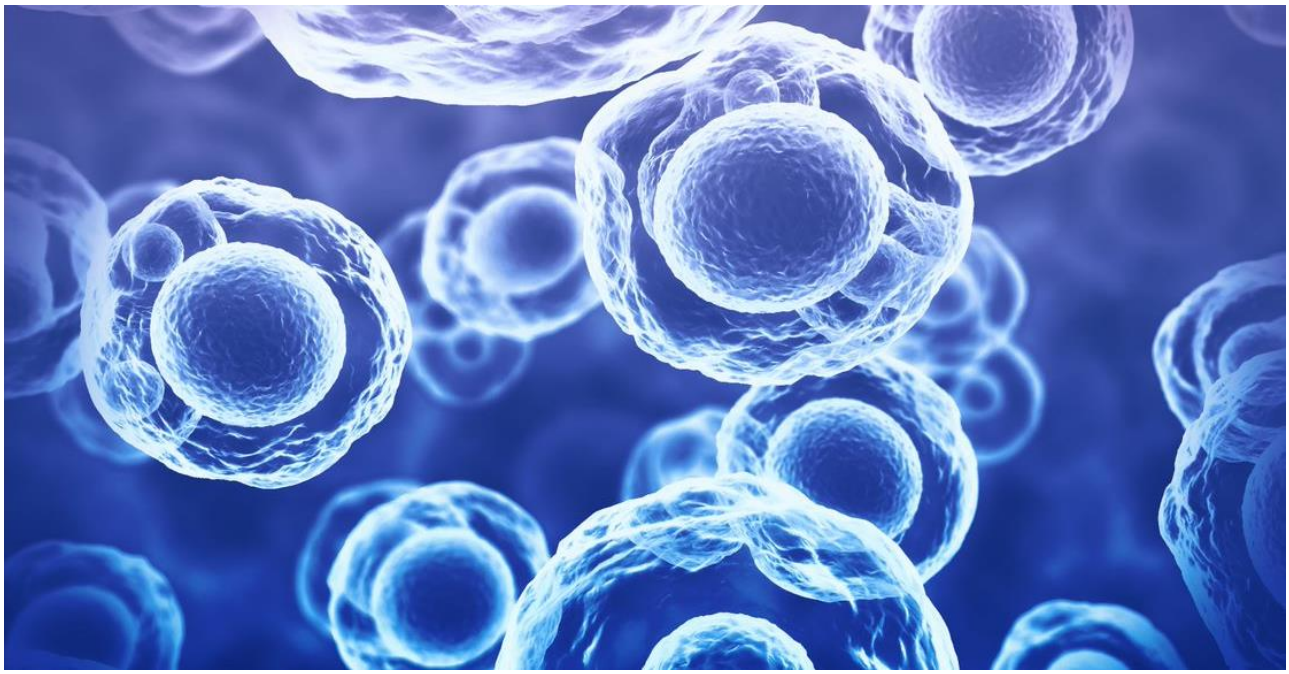
BECKMAN COULTER
Life Sciences

Purifying High Quality Exosomes using Ultracentrifugation



Background

Extracellular vesicles (EVs) are nanoparticles encapsulated by a lipid bilayer. EVs carry a variety of cargoes and play critical roles in intercellular communications and physiological processes. All living cells naturally secrete EVs, which differ in biogenesis and diameter. Exosomes, microvesicles, and apoptotic bodies are all EVs.[1] Exosomes show tremendous promise in the clinic as biomarkers and therapeutics.[2-4]

Challenge

Because exosomes are heterogeneous with respect to size, composition, and function, many of the available techniques for exosome isolation are unsuitable for subsequent characterization and application.[1]

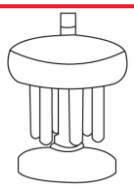
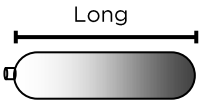
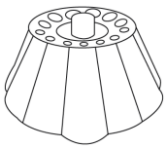
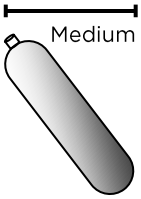
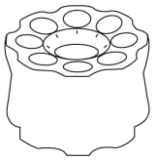
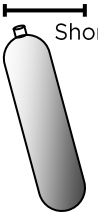
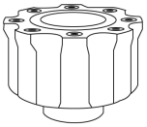
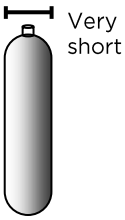
Solution

With the high resolution and separating power of ultracentrifugation (UC), specific subpopulations of exosomes can be reproducibly isolated. UC is the most used EV purification technique and is broadly considered the gold standard.[5,6]

Methods for exosome purification

- (1) Differential ultracentrifugation (DUC) makes use of multiple spins of progressively increasing speeds to first remove contaminants and then pellet exosomes. This step is often followed by further centrifugal purification steps.
- (2) Cushioned DUC adds a 'cushion' of dense material on the bottom of the tube (such as sucrose or iodixanol [OptiPrep™ Density Gradient Medium]) to a typical DUC run. This allows for gentler pelleting and better preserves exosome morphology, integrity, and function.
- Density gradient ultracentrifugation (DGUC) is a high-resolution purification technique used to separate exosomes based on their physical properties (size, shape, mass, and/or density).
    - In (3) isopycnic DGUC, the gradient forming material is added to the sample tube and spun at high speeds until a linear (continuous) density gradient is formed. This causes the exosomes to migrate to the point at which their buoyant density is equal to the surrounding media.
    - (4) Equilibrium zonal DGUC is similar to isopycnic DGUC, but instead of forming a linear gradient, the gradient is layered in specific steps. This results in discrete density boundaries and therefore discrete exosome populations after the run.
    - (5) Rate zonal DGUC employs an initial linear gradient with the sample on top. Once spinning commences, the exosomes begin to sediment at different rates depending on their individual sedimentation coefficients (s-values). In other words, the particles race to the bottom of the tube and the heavier, denser, more globular particles move faster.

Rotors for exosome purification

	Use cases	Angle	Example	Pathlength (at speed)
Swinging-bucket (SW)	With the longest pathlength, SW rotors are the best option available for rate zonal exosome separations. SW rotors are also beneficial for pelleting very small sample masses to maximize visibility and pellet stability.	90°		
Fixed-angle (FA)	Highly versatile, FA rotors are applicable for all exosome purification methods except rate zonal. FA rotors are preferable for larger-scale pelleting, especially with sufficient sample to be visible.	20-30°		
Near-vertical (NVT)	NVT rotors are preferred for density-based separation with less pure samples which may have a small amount of floating or sedimented contaminants.	7-10°		
Vertical (VT)	VT rotors are the most preferable option for density gradient formation and high-resolution density-based separations of exosomes.	0°		

Tubes for exosome purification

Most tubes are available in multiple materials, with polypropylene and Ultra-Clear being the preferred options. Polypropylene tubes afford marginally easier piercing while Ultra-Clear tubes offer improved visibility. When dealing with exosomes, the transparent Ultra-Clear tubes are recommended.

Quick-Seal
Heat-sealed easily and reliably for a robust seal

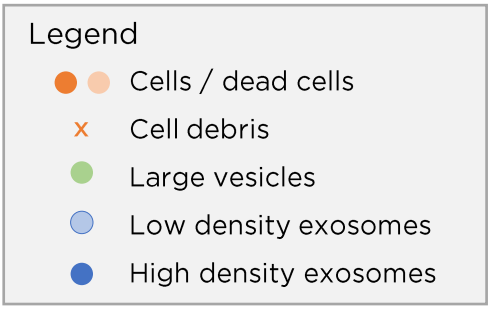
OptiSeal
Plug-based seal provides simplicity and reliability

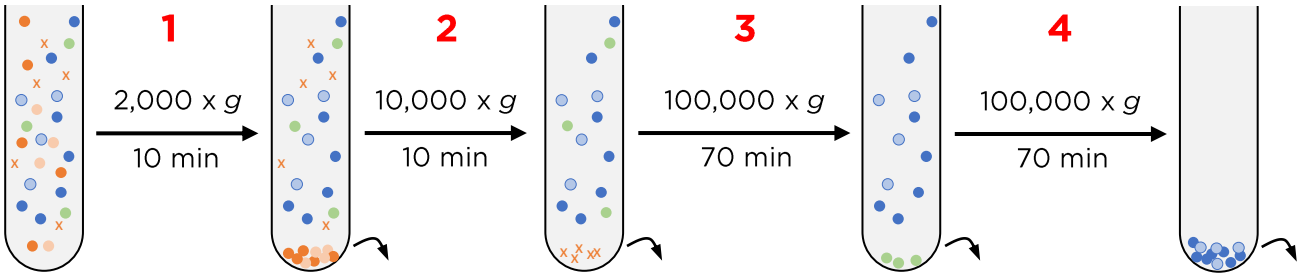
Open Top
Simplest option, no closure at all

Open Top w/ cap
Simple open top with cap assemblies to prevent leakage

Protocols for purifying exosomes

1. Differential ultracentrifugation*





Protocol modified from Xie 2022[7] and Gall 2020.[8]

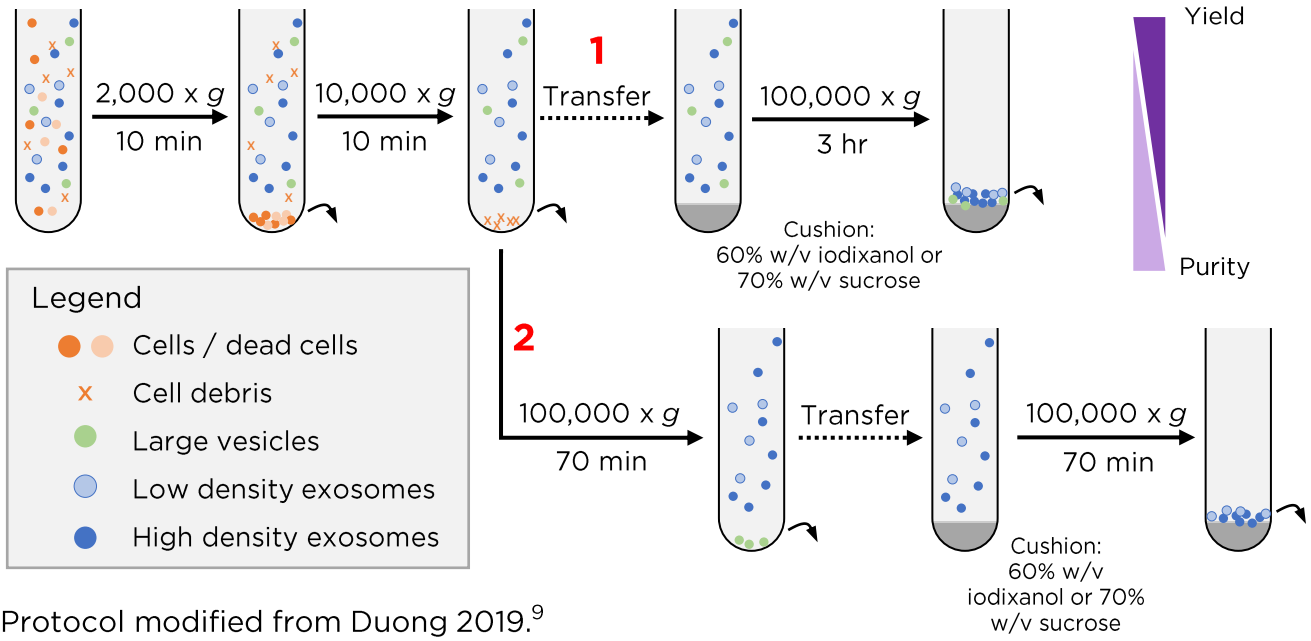
1. Removal of whole cells. Thus, it depends heavily on the exosomes source (e.g., CHO cell culture, blood, etc.). Refer to literature to determine the appropriate speeds to remove specific cell types.
2. Removal of cell debris.
3. Removal of apoptotic bodies and microvesicles.
4. Isolation of exosomes. At this stage, the sample may still contain some larger vesicles but should be enriched for exosomes.

Potential improvements to this protocol:

- Shorter pathlength (better k-factor)
- Conical tubes may provide enhanced pellet recovery

*This protocol can be run in all standard FA and SW rotors.

2. Cushioned differential ultracentrifugation*



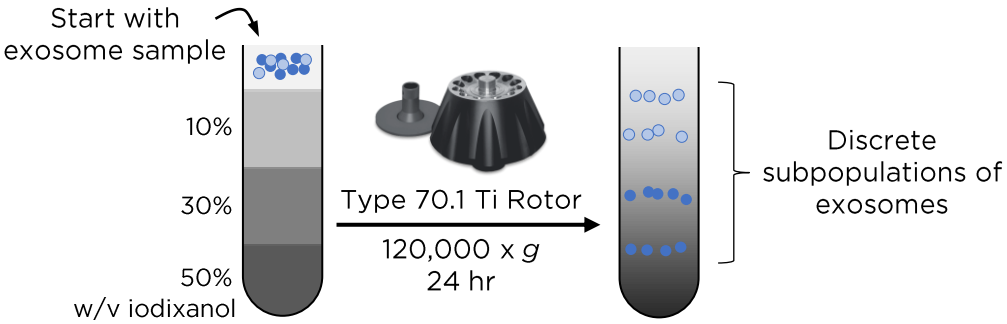
Protocol modified from Duong 2019.[9]

Cushioned DUC is nearly identical to standard DUC, except the exosomes are pelleted on a high-density cushion to better preserve morphology and function. This protocol is a sweet spot in terms of hands-on time and product quality. Notably, two versions are presented, with (1) offering better yield and (2) providing higher purity.

Potential improvements to this protocol:

- Shorter pathlength (better k-factor)

3. Isopycnic density gradient ultracentrifugation†



Protocol modified from Onodi 2018.[10]

In isopycnic DGUC, a crude exosome sample is placed in a tube with a dense material like iodixanol. The tube is spun until equilibrium is reached and a linear density gradient is formed. There are many modifications that can be made including the number of steps,

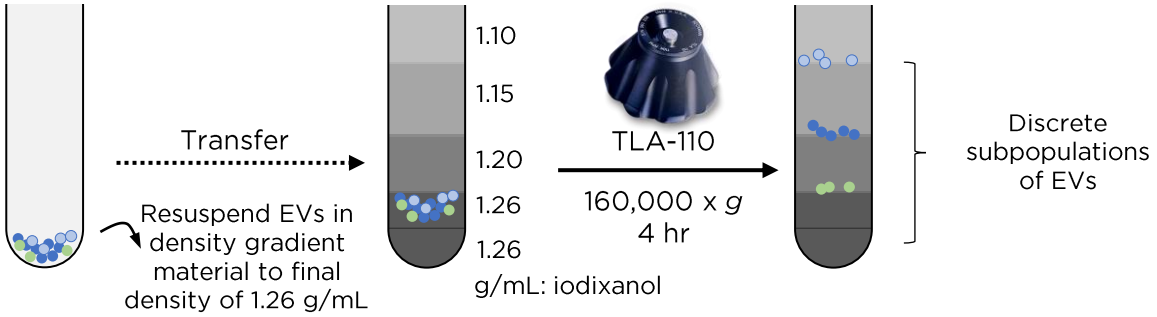
*This protocol can be run in all standard FA and SW rotors. †The speed at which the run can be safely performed is dictated by the starting density and rotor. Please refer to the rotor instructions for use (IFU) to determine safe operating speeds when using dense materials.

volume of each step, position of sample (on top, in between, or below the layers), starting density of sample, run speed and time, and rotor type. Exosome buoyant densities are usually between 1.08 and 1.20 g/mL,[11,12] but this depends on the density gradient forming material used.

Potential improvements to this protocol:

- Use a vertical rotor to shorten the pathlength and reduce run time
- Spin faster to reduce run time*
- Leverage a multispeed protocol to boost efficiency (high speed first to reach equilibrium quickly, then lower speed to flatten the gradient and increase resolution)
- Reduce run time by starting with more layers (trade off with hands-on prep time)

4. Equilibrium zonal density gradient ultracentrifugation

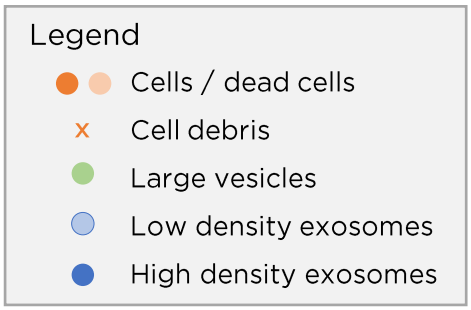


Protocol modified from Iwai 2016.[13]

In equilibrium zonal DGUC, a crude exosome sample is placed in a tube with distinct steps of progressively higher densities. Upon spinning, the exosomes will migrate to the position in the tube equal to their buoyant density, typically at an interface between two steps. Much like isopycnic DGUC, there are many variations of this separation approach. In contrast to isopycnic DGUC, however, the experiment is stopped before a linear density gradient is formed (i.e., the distinct steps are maintained). In this specific example, the exosomes are pelleted in a previous step and resuspended directly into a buffered solution at a specific density, but this is not an absolute requirement.

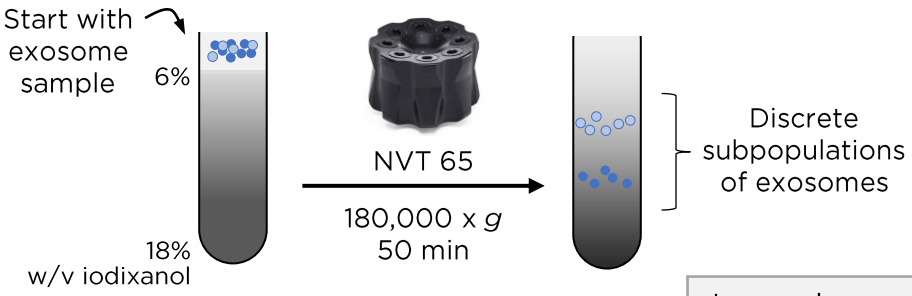
Potential improvements to this protocol:

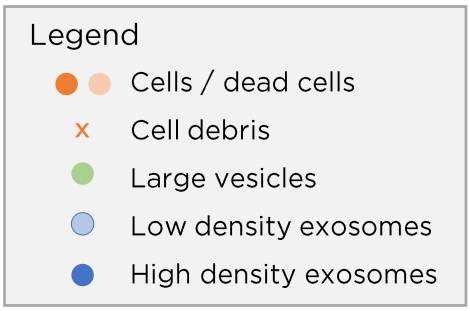
- Use a vertical (or near vertical) rotor to shorten the pathlength and reduce run time
- Spin faster to reduce run time*



*The speed at which the run can be safely performed is dictated by the starting density and rotor. Please refer to the rotor instructions for use (IFU) to determine safe operating speeds when using dense materials.

5. Rate Zonal Density Gradient Ultracentrifugation





Protocol modified from Vaillancourt 2021.[14]

In rate zonal DGUC, a crude exosome sample is placed on top of a preformed linear density gradient. Upon spinning, the exosomes will sediment at different rates depending on their individual sedimentation coefficients.

Potential improvements to this protocol:

- Use a SW rotor to increase the pathlength and boost resolution (this would result in longer run time, however)
- Spin faster to reduce run time*

Choosing the right ultracentrifuge setup for exosome purification†

1. Floor-standing or tabletop?

The Optima MAX-XP has a small footprint while the Optima XE and XPN offer higher capacities and more rotor/tube options

2. Rotor type?

- Fixed-angle (FA)
- Swinging-bucket (SW)
- Vertical (VT)
- Near-vertical (NVT)

MLA-80	MLS-50	MLN-80	Type 70.1 Ti	SW 40 Ti	VTi 65.1
440,000 x *g*	268,000 x *g*	389,000 x *g*	450,000 x *g*	285,000 x *g*	402,000 x *g*
8 x 8 mL	4 x 5 mL	8 x 8 mL	12 x 13.5 mL	6 x 14 mL	8 x 13.5 mL

3. Tube type?

Quick-Seal Open Top	Quick-Seal Open Top	Quick-Seal	Quick-Seal OptiSeal Open Top Open Top w/ cap	Quick-Seal Open Top	Quick-Seal

*The speed at which the run can be safely performed is dictated by the starting density and rotor. Please refer to the rotor instructions for use (IFU) to determine safe operating speeds when using dense materials. †These are only recommended rotors and tubes – please see the Ultracentrifuge Catalog for a complete list of compatible rotors and tubes. Some tubes may require adapters to work with specific rotors.

Reach ultracentrifuge speeds in the Avanti JXN-30 centrifuge

Avanti JXN-30

Cell harvest and lysate clarification

JLA-9.1000
16,780 x *g*
4 x 1000 mL

JLA-12.500
26,890 x *g*
6 x 500 mL

Exosome purification

JA-30.50 Ti
108,860 x *g*
8 x 50 mL

JS-24.15
110,510 x *g*
6 x 15 mL

As part of our high-performance line of centrifuges, the Avanti JXN-30 excels at large volume centrifugation. However, the JXN-30 is unique in that it can also reach the ultracentrifuge speeds required to purify exosomes. Consider the Avanti JXN-30 as a one-stop-shop solution to enable exosome research.

Use the J-LITE® JLA-9.1000 or JLA-12.500 high-capacity rotors to spin down cell cultures, then switch to the JA-30.50 Ti or the JS-24.15 rotors to easily perform exosome isolation.

When the highest versatility is needed, look no further than the Avanti JXN-30.

Comparing ultracentrifuge methods for exosome purification

	DUC	Cushioned DUC	Isopycnic DGUC	Equilibrium Zonal DGUC	Rate Zonal DGUC
Purity					
Yield					
Ease of use					
Run time					
Separation basis	Size and mass (S-value)	Size and mass (S-value)	Buoyant density	Buoyant density	Size and mass (S-value)
Ideal Rotor	FA or SW	FA or SW	VT	VT	SW

The values in this table are meant to serve only as directionally useful estimates using standard protocols.

Please see the relevant centrifuge brochures and rotor manuals (instructions for use) for a complete listing of available instruments, hardware, and consumables.

For more information about Beckman Coulter Life Sciences centrifuges, please visit https://www.beckman.com/centrifuges

For more information about exosomes, please visit https://www.beckman.com/resources/sample-type/extracellular-vesicles/exosomes

References

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2023-GBL-EN-101201-v1