

Exosome Isolation - Ultracentrifugation

Protocol Notes ¹ DRAFT

- This protocol was originally modified from Thery2006, then modified after Laesser2012 and most recently updated to reflect findings in Cvjetkovic2014 reflecting changes in centrifuge rotors and lab equipment.
- This protocol is suitable for serum, BALF, and most other biological fluids.
- If used in medium, FCS must be exosome free. FCS can be replaced with insulin-transferrin-sodium-selenite supplement or BSA (1% w/v) if desired (Thery2002)
- Threads of ultracentrifuge buckets must be coated with Beckman Spinkote, and the gaskets must be coated with Beckman Vacuum grease.
- Must refrigerate 45 Ti rotor buckets at 4 degrees C prior to running.
- Spins are programmed into the L-70k centrifuge for ease of use. Please see page 3-2 of the manual for Programmed Usage.

Biohazard Safety

- Be sure to work in a biohazard safety cabinet at all times. Wipe the cabinet before and after use with 70% ethanol or Bacdown disinfectant.
- Use universal biosafety precautions, wear a lab coat and disposable gloves, and other appropriate PPE.
- When disposing BAL fluids, add bleach to the discarded fluid for 10% bleach solution to disinfect the cells and fluid at least 10 minutes, then discard and rinse down the drain.
- While spinning in centrifuge, cover the samples with the “Biohazard” caps to prevent the spread of infectious content in the case of a spill.
- Dispose trash in the designated “Biohazard” trashcans. Be sure to double bag the pipets before disposal.

Materials

1. 50-mL polystyrene conical tubes (Falcon tubes)
2. Phosphate Buffered Saline (PBS) 1x, prepared on N/A by N/A, location: stored at room temperature in 1-L glass reagent bottle
3. Beckman 45 Ti rotor, serial# 11U 4663 (fixed angle rotor, made in 2011)

¹Created by Steven Poynter on 2012-08-14; Modified by ALG on 2012-10-05; Modified by PI 2013-12-11; Modified by ALG 2014-08-01

4. Beckman Spinkote, Cat# 306812
5. Beckman Vacuum Grease Silicone, Cat# 335148
6. Beckman Centrifuge Tubes, Thinwall, Polypropylene, 94 mL, 38 x 102 mm
 - Cat# 345775
 - Require special caps/spacers for use in 45 Ti rotor
7. Beckman Cap/Spacer Assembly, Aluminum, Tube, 38 mm dia
 - Cat# 330901
 - Requires special neoprene O-rings (see below)
8. Beckman O-Ring or Gasket, Aluminum, 30.0 mm ID x 37.0 mm OD
 - Cat# 346242
 - For use with the aluminum caps/spacers and the thinwall polyal-
lomer/propylene tubes in 45 Ti rotor
9. Beckman Optima L-70k Ultracentrifuge
10. Sorval Legend XTR Centrifuge
11. Sorval rotor F13-14-50cy
12. Ice
13. 0.22 micron cellulose acetate membrane filter

Methods

1. If sample has not yet been centrifuged to remove cells, then centrifuge sample in 50-mL conical tube at 300*g in the Sorval Legend XTR centrifuge for 10 minutes at 4 degrees C.
 1. This is saved as Program 2 on the Sorval Legend XTR centrifuge
 2. *Supernatant* after this step contains the exosomes.
2. Using a serological pipet, remove the supernatant to approximately 2 cm above pellet. Use a pipetman to remove as much remaining supernatant as possible without disturbing the pellet. Transfer supernatant to ultracentrifuge tubes.
 1. Optional: Keep aliquot of supernatant for purification analysis if necessary.
3. Balance ultracentrifuge tubes with cold PBS using a scale.
 1. Fill tubes leaving only a few millimeters of empty space.
 2. Tubes must be weighed within 0.1 – 0.2 g of each other

4. Spin samples in ultracentrifuge, 16,500*g (14,500 RPM with 45 Ti) for 25 minutes at 4 degrees C.
 1. This is saved as Program 4 on the Optima L-70k Ultracentrifuge
 2. *Supernatant* after this step contains the exosomes.
5. Use serological pipet to remove supernatant from each tube, again being careful not to disturb the pellet.
6. Transfer to a 60 mL sterile syringe and filter through 0.22 micron cellulose acetate membrane filter.
7. Transfer supernatant into new ultracentrifuge tubes and replace back in swing buckets. Be sure to lightly re-grease threads of buckets.
 1. Optional: Keep an aliquot of supernatant for purification analysis if desired.
8. Balance tubes with cold PBS as in step 3.
9. Spin in ultracentrifuge 118,000*g (39,000 RPM with 45 Ti) for 5 hours at 4 degrees C.
 1. Requires a *delayed* start in the Ultracentrifuge, timed to complete the spin in the morning when processing can be immediately continued. The holding temperature should be set at 4 degrees C.
 2. The spun pellet cannot sit in the ultracentrifuge for long prior to continuing with processing.
 3. *Pellet* after this step contains exosomes.
10. *Alternative:* If not able to complete processing in the AM which is required by the overnight spin, then spin in ultracentrifuge 118,000*g (39,000 RPM with 45 Ti) for 1.5 hours at 4 degrees C.
 1. This is saved as Program 2 on the Optima L-70k Ultracentrifuge
 2. *Pellet* after this step contains exosomes
 3. Overnight, longer spin is much preferred due to increased yields
12. Again, use a serological pipet to remove the supernatant from the samples. Use a pipetman to remove as much supernatant from the pellet as possible. Resuspend pellet in 60-120 mcL of PBS and store at -80 degrees C.
 1. Optional: If you want to conduct exosomal protein analysis, you can resuspend in lysis buffer (see BCA protocol).

References

1. Théry, C.; Amigorena, S.; Raposo, G. & Clayton, A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. (2006) Current Protocols in Cell Biology;Chapter 3, Unit 3.22: 3.22.1—3.22.29. [Thery2006](#)

2. Théry, C. Zitvogel, L., Amigorena, S., Exosomes: Composition, Biogenesis and Function. (2002) *Nature Reviews: Immunology*;2:569—579. [Thery2002](#)
3. Lässer, C., Eldh, M., Lötval, J. Isolation and Characterization of RNA-Containing Exosomes. (2012) *Journal Visualized Experiments*;59:e3037. [Laesser2012a](#)
4. Cvjetkovic, Aleksander and Lötval, Jan and Lässer, Cecilia, The influence of rotor type and centrifugation time on the yield and purity of extracellular vesicles. (2014) *Journal of Extracellular Vesicles*; 3:23111-23122. [Cvjetkovic2014](#)
5. Beckman Coulter Rotor Conversions [weblink](#)