Exosome Isolation - Ultracentrifugation

Protocol Notes [1]

- 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 SW 41 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) or,

[1] Created 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 SW 41 Ti rotor, serial# 97U 10110 (swing-bucket rotor)
5. Beckman Spinkote, Cat# 306812
6. Beckman Vacuum Grease Silicone, Cat# 335148
7. Beckman Centrifuge Tubes, Thinwall, Polypropylene, 94 mL, 38 x 102 mm
    - Cat# 345775
    - Require special caps/spacers for use in 45 Ti rotor, or:
8. Beckman Polyallomer Centrifuge Tubes, 14 x 89 mm,
    - Cat# 331372
    - These are for the SW 41 Ti rotor (swing bucket type)
9. Beckman Cap/Spacer Assembly, Aluminum, Tube, 38 mm diameter
    - Cat# 330901
    - Requires special neoprene O-rings (see below)
10. 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 polyallomer/propylene tubes in 45 Ti rotor
11. Beckman Optima L-70k Ultracentrifuge
12. Sorval Legend XTR Centrifuge
13. Sorval rotor F13-14-50cy
14. Ice
15. 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. *Alternatively*, use SW 41 Ti (11,500 RPM) 44 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. *Alternatively*, use SW 41 Ti (31,000 RPM) for 6.5 hours at 4 degress 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. *Alternatively*, use SW 41 Ti (31,000 RPM) for 2 hours at 4 degress 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
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3. Lässer, C., Eldh, M., Lötvall, J. Isolation and Characterization of RNA-Containing Exosomes. (2012) Journal Visualized Experiments;59:e3037. Laesser2012a
4. Cvjetkovic, Aleksander and Lötvall, 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