Exosome Isolation - Ultracentrifugation

Protocol Notes [1]

- This protocol was originally modified from Thery2006. In 2013 updated to match methods in Laesser2012a.
- 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
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 SW 41 Ti rotor, serial# 97U 10110 (swing-bucket rotor)
4. Beckman SW 41 Ti 104.9 buckets (6)

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

5. Beckman Spinkote, Cat# 306812
6. Beckman Vacuum Grease Silicone, Cat# 335148
7. Beckman Polyallomer Centrifuge Tubes, 14 x 89 mm, Lot# Z11208SCA, Reorder# 331372
8. Beckman Optima L-70k Ultracentrifuge
9. Jouan CR 412 Centrifuge
10. Ice
11. 0.22 micron cellulose acetate membrane filter

Methods

1. Spin down sample in 50-mL conical tube at 2000xg (3100 RPM in Jouan centrifuge) for 30 minutes at 4 degrees C.
    1. *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 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, 16,500xg (11,500 RPM with SW 41 Ti) for 20 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 120,000xg (31,000 RPM with SW 41 Ti) for 70 minutes at 4 degrees C.

    1. This is saved as Program 5 on the Optima L-70k Ultracentrifuge
    2. *Pellet* after this step contains exosomes

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., Lötvall, J. Isolation and Characterization of RNA-Containing Exosomes. (2012) Journal Visualized Experiments;59:e3037. Laesser2012a