Exosome Isolation - Ultracentrifugation

Protocol Notes

- This protocol was modified from: Current Protocols in Cell Biology

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- 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 (Thery et al. 2002)
- 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)
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. Jouan CR 412 Centrifuge
9. Beckman Optima L-70k Ultracentrifuge
10. Ice

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
4. Spin samples in ultracentrifuge, 12,000xg (8200 RPM with SW 41 Ti) for 45 minutes at 4 degrees C.
    1. This is saved as Program 1 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. 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.

6. Balance tubes with cold PBS as in step 3.

7. Spin in ultracentrifuge 100,000xg (24,000 RPM with SW 41 Ti) for 2 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

8. Use a serological pipet to remove the supernatant from the samples. Use a pipetman to remove as much supernatant from the pellet as possible without disturbing it.

    1. Optional: Keep an aliquot of supernatant for purification analysis if desired.

9. Using cold PBS, condense all pellets from same sample into a clean ultracentrifuge tube and fill up the tube to near maximum volume.

    1. Optional: You can add a 0.2 micron filtration step here, but be sure to use a filter that will not bind exosomes. See supplementary protocols to assess what filters to use.

10. Balance sample tube with another tube filled with PBS.

    1. All swing buckets with screwcaps must be attached to the rotor whether or not they contain sample.

11. Spin in ultracentrifuge, 110,000xg (25,000 RPM with SW 41 Ti) for 1 hour at 4 degrees C.

    1. This is Program 3 on the Optima L-70k Ultracentrifuge
    2. Pellet after this step contains the 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

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