EV-UC200: Exosome Recovery Protocol by Differential Ultracentrifugation (<200 nm)

Technic: Exosome recovery

Goal: EVs Recovery using ultracentrifugation (size-based gradient separation to collect all particles smaller than 200 nm)

Procedure:

1. Collect culture medium. Recover the cell culture medium from the cultured cells and transfer it into centrifuge tubes. Keep samples on ice whenever possible.
2. First centrifugation. Centrifuge the sample at 2000 g for 10 min at 4 °C.
3. Supernatant collection. Carefully collect the supernatant and transfer it into ultracentrifuge tubes.
4. Balancing. Balance the tubes on a scale. Differences of 0.1–0.6 g between tubes were tolerated and deemed acceptable.
5. Second centrifugation. Load the balanced tubes into the ultracentrifuge and spin at 20,000 g (≈14,000 rpm) for 30 min at 4 °C.
6. Supernatant recovery. Carefully collect the supernatant and transfer it into clean Falcon tubes.
7. Filtration. Filter the recovered supernatant through a 0.22 μm filter into fresh ultracentrifuge tubes.
8. Rebalancing. Weigh the tubes again to ensure balance, ideally within 0.1 g difference.
9. Final ultracentrifugation. Spin the tubes at 140,000 g for 1 h 30 min at 4 °C.
10. Supernatant removal. After centrifugation, carefully discard the supernatant without disturbing the pellet.
11. Pellet resuspension. Resuspend the pellet in 60 μL of PBS.

Reagents and Solutions:

- Cell culture
- PBS 1X

Equipment:

- 0.22 μm filter units
- Centrifuge (capable of 2000 g, 4 °C)
- Ultracentrifuge (capable of up to 140,000 g, 4 °C)
- Ultracentrifuge tubes (60 mL)
- Falcon tubes (50 mL)
- Analytical balance (for tube balancing)
- Pipettes and sterile tips

Estimate time: ~ 4 hours