PennVet

EV PURIFICATION FROM CELLS

Starting material:

- Appropriate number of cells to produce at least 150-300mL of conditioned media.
- Cells should be in EV-depleted media. This means that the media is either serum-free or contains serum that has been previously depleted of EVs to avoid contamination with bovine EVs.

Procedure:

1. In hood, collect media with large pipette into 50mL conical tubes. Conditioned media can be combined in tubes from same cell lines.
2. *Trypsinize (~2-4mL for a large plate) one plate of cells for total lysate analysis if desired. Place in incubator for 2-3 minutes and put trypsinized cells in centrifuge to spin and pellet (5min 1000 rpm). Store/process for future analysis as desired.*
3. Place conditioned media tubes into centrifuge and spin 20min @ 2000g.

NOTE: STEPS 1-3 OR 1-2 can be done in own laboratory if desired.

4. Retrieve glass ultracentrifuge tubes (Beckman Coulter) from the refrigerator.
5. Under the hood, transfer supernatant from 50mL conical tubes to chilled ultracentrifuge tubes (Beckman). You can combine supernatants from same cell lines. Discard 50mL tubes (leave 2-3mL at the bottom to ensure you did not disturb the pellet).
6. Take glass tubes and weigh on bench top balance. Equalize masses with PBS for balance (1g = 1mL PBS).
7. Place balanced samples into Beckman Ti45 fixed angle rotor. Be sure rubber stoppers are all in place and close rotor again tightly.
8. Set for 38 minutes (30minute spin with 8 minutes to get started). 9000 rpm (**20,000 x g**) at 4 degrees. Set rotor with samples carefully in the centrifuge, close, and press start when settings are correct. Wait for vacuum to close and reach 450, and the rpm to reach 9000.
9. Get new chilled ultracentrifuge tubes and place in the hood for after the spin.
10. Retrieve samples from the machine. Take care not to disturb the pellet.
11. Under the hood, transfer supernatant from current glass tubes to new glass tubes, careful not to disturb the pellet. Resuspend 20,000 x g pellet in 100ul PBS (pellets from same cell line can be combined in the same 100ul PBS). Store at -80.
12. In the new ultracentrifuge tubes with the supernatant, weigh and balance with PBS. Ultracentrifuge at 30,000 rpm (**100,000 x g**) for 1hour 30min (can be as low as 1 hour or as high as 2 hours) at 4 degrees. Press start and wait for vacuum to close and rpm to reach 25,000 before leaving.
13. Carefully aspirate as much supernatant as possible and re-suspend pellets in PBS. Combine same cell line suspensions into a single tube. Weigh and

balance with PBS. Again ultracentrifuge at 30,000 rpm (**100,000 x g**) for 1hour 30min (can be as low as 1 hour or as high as 2 hours) at 4 degrees. Press start and wait for vacuum to close and rpm to reach 25,000 before leaving.

14. Remove supernatant in the hood and re-suspend large combined pellet in ~100uL PBS and store in -80degrees.