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Isolation of Extracellular Vesicles from Cell Culture Media by Differential Ultracentrifugation

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We use this protocol and it's working

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Abstract

Extracellular vesicles (EVs) are released by all mammalian cells and are thought to be important mediators of intercellular communication. There are many methods for isolating EVs from cell culture media, but one of the most commonly used methods continues to be purification based on ultracentrifugation. This approach has the advantage of allowing a large input volume of cell culture media. Here, we provide a detailed protocol for isolating EVs by differential ultracentrifugation.

Materials

- Cells and cultureware
- Fetal bovine serum (FBS)-depleted media (ultracentrifuge media overnight (16 hours) at 120,000 x *g* to deplete) or media made without FBS such as AIM V media (Gibco)
- PBS without Ca^{2+}/Mg^{2+}
- Ultracentrifuge and rotor
- Polyallomer ultracentrifuge tubes (Beckman Coulter)
- 0.22 µm Steriflip filter tubes (EMD Millipore)
- 50 mL Falcon tubes (Corning)
- Pasteur pipettes
- cOmplete mini protease inhibitor cocktail tablets (Roche)
- 1M HEPES (Gibco)
- HEPES buffer (optional)-20 mM HEPES with protease inhibitor (Every 2 weeks make stock of 200 uL 1x HEPES in 10 mL PBS with 1 cOmplete mini protease inhibitor cocktail tablet. Store at 4 °C.)

1 Culture cells under standard conditions to 50–70% confluency.

Day 1

2 **For suspension cells:** spin down desired total number of cells in six Falcon tubes at 300 x *g* for 5 minutes.

2.1 Aspirate media and resuspend each cell pellet in 40 mL FBS depleted media or media made without FBS. Transfer contents of each Falcon tube to T75 flask and return to incubator.

3 **For adherent cells:** Aspirate media from twelve 15 cm plates.

3.1 Add 20 mL FBS-depleted media or media made without FBS per plate and return cells to incubator.

Day 2

4 After 24 hours, take off all media and divide among 50 mL falcon tubes.

5 Spin at 300 x *g* for 10 minutes at RT to pellet the cells.

6 Transfer supernatant to new 50 mL tubes leaving cell pellet behind.

7 Spin at 2000 x *g* for 10 minutes at RT (to pellet any dead cells).

8 Transfer supernatant to new 50 mL tubes leaving cell pellet behind.

9 Spin supernatant at 16,500 x *g* for 20 minutes at 4 °C (to pellet large EVs).

10 Transfer supernatant to new 50 mL tubes, leaving pellet behind.

11 Pass supernatant through Steriflip 0.22 µm filter.

12 Transfer supernatant to polyallomer ultracentrifuge tubes. Centrifuge at 120,000 x *g* (26,500 RPM with SW32Ti rotor) for 70 minutes at 4 °C.

13 Remove most of supernatant, leaving ~2 cm of media above pellet. Add 5 mL PBS to each tube. Vortex on medium speed for a few seconds. Fill to top of each tube with PBS.

14 Again, centrifuge at 120,000 x *g* for 70 minutes at 4 °C.

15 Aspirate all of supernatant with Pasteur pipet without touching bottom of tube where pellet is located.

16 Resuspend pellet either in PBS or directly in the desired lysis buffer for western blot. If storing for later use, resuspend in HEPES buffer, and store in -80 °C.