Title

Protein collection and isolation from cells

Protocol

Materials:

- Box with ice
- Phosphate Buffered Saline (PBS) (Cat. No: D8537, Sigma-Aldrich)
- Protease Inhibitor Cocktail (PIC) (Cat. No.: P8340, Sigma-Aldrich)
- 5mM Tris-HCl
- Tissue culture dish with cells
- 1,5 ml tubes

Procedure:

1. Aspirate medium and wash 3x with pre-warmed PBS.
2. Aspirate volume until completely dry.
3. Make PBS + PIC solution 100:1 and add a sufficient volume of solution to fully cover the culture ware surface (e.g. 500µl for 10cm dish).
4. Scrape off cells.
5. Put cells into a 1.5 ml tube, pipette up and down a few times to make sure that all the cells are detached.
6. Place tubes on ice.
7. Centrifuge for 5min, 300 x g, 4°C
8. Aspirate supernatant carefully, do not aspirate the pellet containing the cells.
9. Re-suspend the pellet in Tris-HCl / PIC (100:1).
    1. Chose the volume of Tris-HCl/ PIC depending on the size of the pellet (for a 10 cm dish normally between 200 and 300 µl).
10. Mark the tubes with cells with: date, passage number and a “protein” text.
11. Storage at -80°C until further processing.
12. 5 freeze (liquid nitrogen)-thaw (37°C water bath) cycles, vortex after every thaw-step and check the labeling (the freezing passage can delete labeling).
    1. Put samples into liquid nitrogen for 10 seconds and transfer it to the water bath until it is liquid.
13. 2x3 strokes with ultrasonic processor.
    1. Between the stroke-cycles leave a short break.
14. Transfer 25-50 µl in 1.5ml tube —> **total protein.**
15. Centrifuge the remaining volume at 4°C, 800xg for 5 min.

16. Transfer the supernatant into a new 1.5ml tube (label: membrane protein), they should have the same weight, check with balance!
    1. Tip: if you transfer the same volume of each sample, the weight should be the same.
    2. Balance weight with Tris-HCl.
17. Cool down the ultracentrifuge to 4°C.
18. Ultracentrifugate your samples at 4°C, 100 000xg for 45 min (check the ultracentrifuge after 15min if everything is ok).
19. Transfer the supernatant into a new 1.5ml tube —> **cytosolic protein.**
20. Suspend your pellet in 50 µl Tris-HCl by pipetting up and down several times before 2x3 strokes with ultrasonic processor —> **membrane protein.**