Exosome Extraction

Introduction

This is the protocol for exosome extraction from cell media.

Material

- Cultured cell at 80% of confluency.
- PBS
- Centrifuge machine at 2K, 10k and 100K g.

Process

Step 1. Take the medium from each cells cultures in flask T175 and place it in 4 Falcon tubes 50 ml.

Step 2. centrifugate at 2000 g for 20 min which allows sedimentation of cell debris and large vesicles like apoptotic bodies in order to remove them.

Step 3. Take the medium again and put it in a 50 ml falcon tube and centrifugate at 10 000 g for 30 min. This step separates microvesicles.

Step 4. Put the medium in 6 godet 32ml max and centrifugate at 100.000 g for 1h15 min to obtain smaller extracellular vesicles, including exosomes. Don't forget to balance the weight at 0.01g

Step 5. Get rid of the medium with pipette Pasteur by aspiration, be careful and let some media at the bottom, a line can be visible (where we have the transparent exosomes).

Step 6. Mix the bottom of each tube and Put all of the samples in one or two tubes of godet.

Step 7. Complete the tube with PBS until 32 ml.

Step 8. Centrifuge at 100.000 g for 1h15 to eliminate smaller contaminant residues from the exosome and obtain pure exosomes.
Step 9. Get rid carefully of the medium with pipette Pasteur and let some until the line (where we have the exosomes) and then empty the tube by slowly inclinate it.

Step 10. Resuspend the exosome with 50 µl of PBS (scratch the wall of the tube)

Step 11. Put them with another 150 µl of PBS (total 200 µl) in -20°C