Protocol

Protocol for Isolation of Extracellular Vesicles from Human Blood Plasma

1. Dilute 1 mL of blood plasma with an additional 1 mL of phosphate-buffered saline (PBS).
2. Centrifuge the sample at low speed (1000-2000 x g) for 5 minutes to separate the plasma from the blood cells.
3. Remove the plasma and transfer it to a new tube.
4. Centrifuge the plasma at high speed (12,000 x g) for 20 minutes to pellet larger cell debris
5. Remove the supernatant and transfer it into ultracentrifuge tubes.
6. Ultracentrifuge the plasma (100,000 x g) for 2 hours minimum to pellet the extracellular vesicles (EV).
7. Carefully drain the ultracentrifuge tubes without disturbing the EV pellets.
8. One ultracentrifuge tube at a time, resuspend the pellet in a suitable buffer, such as phosphate-buffered saline (PBS), and transfer it to a new tube.
9. Centrifuge the resuspended pellet again at high speed to pellet the EVs.
10. Remove the supernatant and resuspend one of the EV pellets in a small volume of PBS or sterile water (scrape the pellet with a thin pipette to dislodge).
11. Transfer the PBS containing the resuspended to a new ultracentrifuge tube and repeat.
12. The resulting solution should contain the purified EVs.