

Iodixanol gradient separation of Exosomes from Cells or Plasma:

1. Make discontinuous gradient (layer carefully 30, 10, 10% iodixanol solutions) in 3-4mL ultracentrifuge tube:
 - a. Bottom (contains EV sample): 30% iodixanol (1.6mL total): 800ul of 60% iodixanol/Optiprep stock PLUS 800ul of buffer mixed with EV pellet.
 - b. Middle: 20% iodixanol (700uL total): 233uL of 60% iodixanol stock + 467uL buffer
 - c. Top: 10% iodixanol (700uL total): 117uL of 60% iodixanol stock + 583uL buffer

**Buffer recipe: 0.25M sucrose, 10mM Tris pH 8.0, 1mM EDTA (pH 7.4)

2. Spin at 350,000g (54,000 rpm for SW 55 Ti rotor), 4°C, for 70 min with max deceleration (no. 9).
3. 10 fractions of 260µL are then collected starting from top of the tube. Separately, aliquot 10uL of each fraction for density analysis. Density is assessed with a refractometer.
4. Add 1mL PBS to all fractions and centrifuge (BECKMAN Optima TLA Ultracentrifuge in a TLA-100.2 rotor at 100,000g (53,000 rpm), 4°C for 70 min.
5. Discard supernatant and resuspend in 1ml PBS and again centrifuge (BECKMAN Optima TLA Ultracentrifuge in a TLA-100.2 rotor) at 100,000g (53,000 rpm), 4°C for 70 min
6. Discard supernatant and resuspend pellets from each fraction in 60-80uL PBS and transferred to -80°C for longer storage.