Exosome isolation from serum

1. Thaw human blood serum on ice (1.5 h)
2. Place the required number of iZON columns in qEV rack and equilibrate the columns to room temperature (1.5 h)
3. Cool down the Centrifuge 5424 R to 4 °C.
4. Centrifuge serum at 1,500 x g for 10 min at 4 °C.
5. Transfer a clear supernatant into a new microcentrifuge tube and centrifuge at 10,000 x g for 20 min at 4 °C.
6. Transfer a clear supernatant into a new microcentrifuge tube and place the tube on ice.
7. Remove the top cap of iZON column carefully and place the column in the rack.
8. Remove the bottom cap and allow buffer to start running through the column
9. After the storage buffer stops flowing, flush the column with 2 x 2 ml of 1X PBS. Do not allow the column to run dry! (Empty the waste tube when it is full.)
10. Load 150 µl of serum on the column and allow it to run into the column.
11. Load 800 µl of 1X PBS on the column and wait for the column to stop flowing.
12. Place 1.5 ml (0.5 ml) Eppendorf tube under the column in the holder.
13. Load 500 µl of 1X PBS on the column, start collecting exosomes and wait for the column to stop flowing.
14. Place the 500-µl exosomal fraction on ice.
15. Before storing samples on -80 °C proceed with enzymatic treatments
16. For the analysis: aliquot 150 µl of exosomal fraction to a clean 0.5 mL tube for DLS analysis and 10 µl for TEM analysis.

Exosomal RNA extraction

Exosomal fraction treatment before RNA extraction

1. Pre-heat a thermomixer to 37 °C.
2. Add 10 µl of proteinase K to the exosomal fraction, gently vortex, spin down shortly, and incubate at 37 °C for 10 min while shaking at 300 rpm.
3. Add 5 µl of RNase to the exosomal fraction, gently vortex, spin down shortly, and incubate at 37 °C for 10 min while shaking at 300 rpm.
4. Add 4 µl of RNase inhibitor, gently vortex and spin down shortly.
5. Place the exosomal fraction on ice or alternatively store on -80 °C.