Capital Biosciences
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Urine SubX™ Exosome isolation kit
(large scale)

Catalog No. EXUR-1000

For Research Use Only

Product Description

Urine SubX™ Exosome isolation kit is designed for isolation of exosomes urine. Our technology is based on the use of a proprietary bi-functional substance (SubX™) that binds exosomes under physiological conditions (e.g. directly in biological liquids. The unique property of SubX™ is that both ends of the molecule bear lipid binding groups. This feature allows each molecule of SubX to anchor two exosomes (i.e. dimerize). Excess of SubX™ in the solution results in aggregation of up to 10-15 exosomes. Kit is designed to provide enough reagents for exosome isolation from up to 250 ml urine, i.e. 5 isolations from 50 ml samples or 10 isolations from 25 ml samples.

Kit components, shipping and storage conditions:

- **Urine Stabilization Solution** (0.5 ml) Ready-to-use. Store at room temperature.
- **SubX™ Solution** (35 ml). Ready-to-use. Store at room temperature
- **Wash-Ex Solution** (5 ml) Ready-to-use. Store at room temperature in a dark place.
- **Exosome Reconstitution Buffer (ERB)** - 5 ml. Store at room temperature.

Kit is shipped in ambient conditions.

Required materials (not supplied):

Thermo Scientific™ Nalgene™ Oak Ridge High-Speed PPCO Centrifuge Tubes (Catalog No.05-529-1D)

General Precautions

All biological samples should be considered as potentially infectious. Proper biosafety measures should therefore be carried out when using this kit.

Protocol

Preparation of Cell-free Urine Sample:

1. Collect urine in 50 ml falcon tube
2. Add 10 µl **Urine Stabilization Solution** per 5 ml of urine
3. Centrifuge at 200 x g (~1,000 RPM) for 10 minutes to remove urine exfoliated cells and debris. Decant cell free urine into a new 15-50 mL conical tube.
4. Centrifuge the cell-free urine at 1,800 x g (~3,000 RPM) for 10 minutes to remove any residual debris or bacterial cells.
5. Transfer cell-free urine into a fresh 15-50 mL conical tube. Cell-Free Urine is now ready for Exosome purification.
6. For exosome isolation transfer cell-free urine into a fresh 40 ml Nalgene centrifugation tube.

Exosome isolation:

1. For exosome isolation transfer cell-free urine into a clean 40 ml Nalgene centrifugation tube (e.g., 2x 25 ml)
2. Add **0.7 ml SubX™** per 5 ml supernatant, mix and incubate 30-40 min at room temperature
3. Centrifuge 10 min at 10,000 rpm (~14,000 g) in high-speed centrifuge
4. Discard supernatant, invert tube onto paper towel and let remaining liquid to drain
5. Add 300 µl of **Wash-Ex** buffer to the pellet. Resuspend the pellet by pipetting and transfer it into 1.5 ml Eppendorf tube.
6. Centrifuge the tube for 5 min at 14,000 rpm and discard supernatant.
7. Resuspend pellet in **0.2-0.5 ml ERB** (**Exosome Reconstitution Buffer**)
8. Centrifuge at 14000 rpm for 5 min to pellet [**SubX-DNA**]complex.
9. Aspirate **Exosome supernatant** into new tube. It is ready to use.
10. For long-term storage freeze at -80C
11. Optional: remaining insoluble pellet may be used later for isolation of cfDNA.