Capital Biosciences
innovative solutions for life sciences

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SubX™ Exo-Plasma isolation kit

INSTRUCTION MANUAL (v1.2)

Catalog No. EXPL-0100
For Research Use Only

Product Description

SubX™ Exo-Plasma kit is designed for isolation of exosomes from plasma or serum. Our technology is based on the use of 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™ molecules in the solution results in oligomerization of up to 10-15 exosomes and formation of micron-size particles that are easily precipitated in a brief 14K x g centrifugation step. A specially designed buffer allows for reconstitution of the pelleted exosomes back to free monomer format suitable for downstream applications. Universal conditioning buffer makes our kit compatible with all tested commercial serum/plasma storage systems/solutions.

Kit components, shipping and storage conditions:

Plasma Condition Solution (2x 1.5 ml) Ready-to-use. Store at room temperature.
SubX™ Solution (2x7 ml). Ready-to-use. **Store refrigerated +4°C.**
Binding Matrix (1 ml). Ready-to-use. Store at room temperature in a dark place.
Exosome Reconstitution Buffer (ERB) – 2x 7 ml. Store at room temperature.

Kits are shipped at ambient temperatures.

Important Notes (Read this first!!!)

- To prevent contamination of circulating cell free DNA with cellular DNA centrifuge plasma for at least 1-2 min at 14000 rpm before use, carefully aspirate supernatant not to disturb possible cell debris pellet and transfer into new tube.
- If working with plasma from **EDTA blood** collection tubes, e.g. Streck Cell-Free DNA BCT™, it is important to add **Plasma Condition Solution** (1/30 volume) before adding SubX™ Solution.
- We recommend using Posi-Click 1.5 ml Eppendorf tubes (Denville Scientific, Inc., Cat. C2170) to prevent material loss during vortexing due to possible cap opening.

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Exosome Isolation from 1 ml or 2 ml plasma/serum.
(**100 isolations**)

1. Add **30 µl Stabilization (Plasma Condition) Solution** to 1 ml plasma or **60 µl** to **2 ml** of plasma and vortex for 5 seconds.
2. Add **140 µl** of **SubX™** solution to **1 ml** of human plasma or **280 µl** of **SubX™** solution to **2 ml** of human plasma, close the tube and immediately vortex for 10-15 seconds. Turbidity may occur. Incubate 3 min at room temperature; vortex 3-4 times during incubation.
3. Vortex **Binding Matrix (BM)** for 15 sec; open the tube and add **10 µl** of **BM** slurry to **SubX™**-plasma mixture, close the tube and incubate 3 min at room temperature; briefly (~10 sec) vortex 3-4 times during incubation.
4. Centrifuge the tube for 5 min at 14,000 rpm, aspirate the supernatant carefully by pipetting taking care not to disturb the **pellet**. DynaMag™ magnet holder (if available) can be used in combination with centrifugation to make it easier to aspirate the supernatant.
5. (**Optional**) Wash the pellet with 100 µl **SubX-ERB** solution **[90µl ERB+10µl SubX] pre-mixed in a separate tube**.
   NB! Do not add 90µl ERB to the pellet followed by 10µl SubX addition!
6. Centrifuge the tube for 1 min at 14,000 rpm.
7. Aspirate the supernatant carefully by pipetting taking care not to disturb the **pellet**. DynaMag™ magnet holder can be used after centrifugation to make it easier to aspirate supernatant.
8. Resuspend the **pellet** in **0.1-0.2 ml ERB** and reconstitute exosomes for 20-30 min at room temperature.
9. Centrifuge the tube for 1 min at 14,000 rpm.
10. Aspirate the supernatant carefully by pipetting taking care not to disturb the **pellet** and transfer into fresh tube. (**Optional**) Pellet can be saved and used later for cfDNA isolation.
11. Keep the exosome containing supernatant at +4°C for next-day use or at -20°C for long-term storage.

Notes:

Step 3 can be skipped of cfDNA isolation is not required. However, BM enhances precipitation of the SubX-Exosomes aggregates.

Incubation duration time can be extended if necessary.

Although Binding Matrix is based on magnetic beads centrifugation is recommended to collect all liquid from the tube cap.

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Alternative Exosome Isolation Protocol from fresh plasma.

1. Dilute **1 ml** plasma with 19 ml PBS (1:20) and vortex for 5 seconds.
2. Centrifuge consequently at 500g, then at 2,000g and finally at 10,000g to remove cell debris and large membrane vesicles.
3. Transfer supernatant into fresh tube for exosome precipitation.
4. Add **140 µl** of **SubX™** solution, close the tube and immediately vortex for 10-15 seconds.
5. Vortex **Binding Matrix (BM)** for 15 sec; open the tube and add **10 µl** of **BM** slurry to **SubX™**-plasma mixture, close the tube and incubate 3 min at room temperature; briefly (~10 sec) vortex 3-4 times during incubation.
6. Centrifuge the tube for 5 min at 14,000 rpm, aspirate the supernatant carefully by pipetting taking care not to disturb the **Pellet.**
7. Discard supernatant
8. Reconstitute exosomes in **100 µl ERB** and incubate at room temperature for 30 min
9. Centrifuge at 10,000g
10. Transfer supernatant into fresh tube and store at -20°C until further use.

Exosome Isolation from 0.1 ml of plasma:

1. **Dilute 0.1 ml of fresh plasma with 1.9 ml PBS**
2. **Centrifuge diluted plasma at 10,000rpm for 30 min to remove cell debris.**
3. **Transfer supernatant to fresh Eppendorf tube and add 10 µL SubX™ (1/10 of the plasma initial volume), vortex;**
4. Vortex **Binding Matrix (BM)** for 15 sec; open the tube and add **5 µl** of **BM** slurry to **SubX™**-plasma mixture;
5. 30 min incubation at room temperature to form the EV aggregates;
6. Precipitation of EV aggregates by centrifugation at 10,000 g for 30 min;
7. EV disaggregation in 20-50 µL exosome reconstitution buffer (ERB) for 20-30 min.