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Exosome and DNA Isolation from 1 ml or 2 ml plasma/serum. (up to 50 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 **200 µl** of **SubX™** solution to **1 ml** of human plasma or **400 µl** of **SubX™** solution to **2 ml** of human plasma, close the tube and immediately vortex for 10-15 seconds. Turbidity may occur. Incubate 3-5 min at room temperature; vortex 3-4 times during incubation.
3) Vortex **Binding Matrix (BM)** for 15 sec; open the tube and add **20 µl** of **Binding Matrix** slurry to **SubX™**-plasma mixture, close the tube and incubate 3-5 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 **Binding Matrix**, discard supernatant.
5) Discard supernatant, invert tube onto paper towel and let remaining liquid to drain or aspirate carefully by pipette
6) Wash [BM-SubX] pellet with 200 µl of **Wash-Ex** buffer
7) Centrifuge the tube for 3 min at 14,000 rpm and discard supernatant
8) Resuspend pellet in **0.1-0.2 ml ERB** (**Exosome Reconstitution Buffer**)
9) Centrifuge at 14000 rpm for 1-5 min to pellet [SubX-DNA-BM]
10) Transfer Exosome supernatant into new tube and freeze
11) Resuspend [SubX-DNA-BM] pellet in **0.5 ml Wash-1** solution, mix and incubate 3-4 min
12) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
13) Add **0.5 ml Wash-G** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;
14) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
15) Add **0.6 ml Wash-2** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;
16) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
17) Add **0.6 ml Wash-2** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;
18) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
19) **ESSENTIAL step!** Briefly centrifuge the tube for 15 sec at 14,000 rpm to remove residual traces of washing buffer from the tube wells, then aspirate traces of Wash-2 solution by using a 10-20 µl micropipette tip and discard.
20) Add 30-50 µl of **DNA Elution Buffer** to the pellet, tightly close the tube and incubate at +60°C for 5 min. Briefly (~10 sec) vortex the tube 5-6 times during incubation.
21) Centrifuge the tube for 1 min at 14,000 rpm, carefully aspirate the cfDNA containing supernatant, transfer to a new tube and **save**.
22) (**Optional**) Repeated elution can yield additional 10-20% of DNA.
23) Keep the cfDNA containing supernatant at +4°C for next-day use or at -20°C for long-term storage.

DNA recovery: Our kit allows for isolation of DNA fragments under 50 bp and even less. Typical DNA recovery ranges from 1 to 100 ng/ml of plasma. The yield varies depending on the sample source and donor health conditions.

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SubX™ Exo-cfDNA isolation kit

INSTRUCTION MANUAL (v1.5)

Catalog No. EXOD-0050
For Research Use Only

Product Description

SubX™ Exo-cfDNA kit is designed for isolation of exosomes and circulating cell-free DNA (cfDNA) directly from liquid biopsies (plasma or serum) without Proteinase K. Our technology is based on the use of proprietary bi-functional substance (SubX™) that binds DNA under physiological conditions (e.g. directly in biological liquids), followed by adsorption of [DNA- SubX™] complex on a solid phase matrix. 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. Since SubX™ captures DNA via phosphate residues (groups) it allows for elimination of bias related to both AT/CG content and DNA fragments length, thus improving extraction efficacy and accuracy of downstream applications. All currently available commercial kits are [silica-chaotropic salt]-based and exhibit bias for either short or long DNA fragments, as well as for the GC content. In addition, such systems suffer from lot-to-lot minor surface differences of silica that results in variations of extraction efficiency of small DNA fragments. Our approach eliminates these problems. Specially designed solid-phase matrix does not require addition of exogenous RNA to prevent non-specific DNA adsorption. 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.
Urine Condition Solution (0.5 ml) Ready-to-use. Store at room temperature.
SubX™ Solution (2x7 ml). Ready-to-use. Store at room temperature
Wash-Ex Solution (10 ml) Ready-to-use. Store at room temperature in a dark place.
Exosome Reconstitution Buffer (ERB) - 5 ml. Store at room temperature.
Binding Matrix (1 ml). Ready-to-use. Store at room temperature in a dark place.
Wash-1 Solution (30 ml). Ready-to-use. Store at room temperature in a dark place.
Wash-G Solution (10 ml)**. Add 15 ml of 96% molecular grade ethanol before use.**
Wash-2 Solution (2x11 ml). **Add 55 ml of 96% molecular grade ethanol before use.**
Elution Buffer: (2x1.5 ml); Ready-to-use. Store at room temperature.

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. One can skip step 1 if working with plasma from **citrated blood** collection.
- Optimal results are achieved when Wash-G and Wash-2 solution is diluted with molecular grade 96% ethanol.
- We recommend using Posi-Click 1.5 ml Eppendorf tubes (Denville Scientific, Inc., Cat. C2170) to prevent material loss during vertexing due to possible cap opening.
- Binding Matrix can be separated from the liquid phase with magnetic stand such as DynaMag™-2 Magnet. **Very important: always pellet Binding Matrix by centrifugation after step 3 of the protocol.** If using magnetic separation protocol, centrifugation for a short time is necessary to collect all materials from the tube cap.

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DNA and exosome isolation protocol from large volume of urine:

1) Collect urine in 50 ml falcon tubes
2) Add 10 µl **Urine Condition Solution per 5 ml of urine**
3) Centrifuge 15 min at 3500 rpm (~2400 g) to pellet cell debris
4) Transfer supernatant into fresh 40 ml Nalgene centrifugation tubes (e.g., 2x 25 ml)
5) Add **1 ml SubX™** per 5 ml supernatant, mix and incubate 30-40 min at room temperature
6) Add **BM** (**30 µl**), mix and incubate 3-4 min
7) Centrifuge 10 min at 1000 rpm (~14000 g) in high-speed centrifuge
8) Discard supernatant, invert tube onto paper towel and let remaining liquid to drain
9) Wash [**BM-SubX**] pellet with 300 µl of **Wash-Ex** buffer. Resuspend the pellet by pipetting and transfer into 1.5 ml Eppendorf tube.
10) Centrifuge the tube for 5 min at 14,000 rpm and discard supernatant
11) Resuspend pellet in **0.2-0.5 ml ERB** (**Exosome Reconstitution Buffer**)
12) Centrifuge at 14000 rpm for 5 min to pellet [**SubX-DNA-BM**]
13) Aspirate Exosome supernatant into new tube and freeze at -80C
14) Resuspend [**SubX-DNA-BM**] pellet in **0.5 ml Wash-1** solution, mix and incubate 3-4 min
15) Centrifuge for 1 min at 14000 rpm
16) Discard supernatant
17) Resuspend [**SubX-DNA-BM**] pellet in **0.5 ml Wash-G** solution, mix and incubate 3-4 min
18) Centrifuge for 1 min at 14000 rpm
19) Discard supernatant
20) Resuspend pellet in **0.8 ml Wash-2**, mix and incubate 3-4 min
21) Centrifuge for 1 min at 14000 rpm
22) Discard supernatant
23) Resuspend pellet in **0.8 ml Wash-2**, mix and incubate 3-4 min
24) Centrifuge for 1 min at 14000 rpm
25) Discard supernatant
26) Centrifuge 15 sec at 14000 rpm
27) Aspirate leftover using 10-20 µl tip
28) Resuspend pellet in **50 – 200 µl DNA Elution Buffer**
29) Elution: Incubate at 60^{0}C for 5 minutes at 1200 rpm on Thermomixer
30) Centrifuge at 14000 rpm for 1 min
31) Aspirate DNA supernatant and transfer to new tube and fr

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DNA Isolation from exosomes.

1. Dilute **0.1 ml** exosomes with **0.9 ml** PBS.
2. Add **140 µl** of **SubX™** solution, close the tube and immediately vortex for 10-15 seconds.
3. Incubate **30 min** at room temperature; vortex 3-4 times during incubation.
4. Add **BM** (**10 µl**), mix and incubate 3-4 min
5. Centrifuge at 10,000g for 5 min
6. Discard supernatant
7. Resuspend [**SubX-DNA-BM**] pellet in **0.3 ml Wash-1** solution, mix and incubate 3-4 min
8. Centrifuge for 1 min at 14000 rpm
9. Discard supernatant
10. Resuspend [**SubX-DNA-BM**] pellet in **0.3 ml Wash-G** solution, mix and incubate 3-4 min
11. Centrifuge for 1 min at 14000 rpm
12. Discard supernatant
13. Resuspend pellet in **0.6 ml Wash-2**, mix and incubate 3-4 min
14. Centrifuge for 1 min at 14000 rpm
15. Discard supernatant
16. Resuspend pellet in **0.6 ml Wash-2**, mix and incubate 3-4 min
17. Centrifuge for 1 min at 14000 rpm
18. Discard supernatant
19. Centrifuge 15 sec at 14000 rpm
20. Aspirate leftover using 10-20 µl tip
21. Resuspend pellet in **50 – 200 µl DNA Elution Buffer**
22. Elution: Incubate at 60^{0}C for 5 minutes at 1200 rpm on Thermomixer
23. Centrifuge at 14000 rpm for 1 min
24. Aspirate DNA supernatant and transfer to new tube and freeze.

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