Capital Biosciences
innovative solutions for life sciences

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SubX™ Urine cfDNA isolation kit

INSTRUCTION MANUAL (v1.5)

Catalog No. URDNA-0100
Up to 100 isolations.

For Research Use Only

Product Description

SubX™ kit is designed for isolation of circulating cell-free DNA (cfDNA) directly from urine 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. 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.

Kit components, shipping and storage conditions:

- **SubX™ Solution** (2 x 10 ml). Ready-to-use. **Store refrigerated +4°C.**
- **Urine Condition Solution (1 ml)** Ready-to-use. Store at room temperature.
- **Binding Matrix** (2 ml). Ready-to-use. Store at room temperature in a dark place.
- **Wash-1 Solution** (2x25 ml). Ready-to-use. Store at room temperature in a dark place.
- **Wash-G Solution** (20 ml)**. Add 30 ml of 96% molecular grade ethanol before use.**
- **Wash-2 Solution** (2x 11 ml). **Add 55 ml of 96% molecular grade ethanol before use.** Store at room temperature in a dark place.
- **Elution Buffer**: (4x1.25 ml); Ready-to-use. **Store refrigerated +4°C.**

Kits are shipped at ambient temperatures.

Important Notes

- To prevent contamination of circulating cell free DNA with cellular DNA centrifuge urine 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.
- Optimal results are achieved when 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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MAIN Protocol:

DNA Isolation from 1 ml or 2 ml urine.
(50 isolations)

1) Add **2 µl Condition Solution** to 1 ml urine or **4 µl** to **2 ml** of urine and vortex for 5 seconds.
2) Add **200 µl** of **SubX™** solution per **1 ml** of urine, close the tube and immediately vortex for 10-15 seconds. Turbidity occurs. Incubate 3 min at room temperature; vortex 3-4 times during incubation.
3) Vortex **Binding Matrix** for 15 sec; open the tube and add **20 µl** of **Binding Matrix** slurry to **SubX™-urine** 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 1 min at 14,000 rpm, aspirate the supernatant carefully by pipetting taking care not to disturb the **Binding Matrix**, discard supernatant.
5) Add **0.5 ml Wash-1** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;
6) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
7) Add **0.5 ml Wash-G** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;
8) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
9) Add **0.6 ml Wash-2** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;
10) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
11) Add **0.6 ml Wash-2** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;
12) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
13) Add **0.6 ml Wash-2** 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) **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.
16) Add 30-50 µl of **Elution** solution 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.
17) Centrifuge the tube for 1 min at 14,000 rpm, carefully aspirate the cfDNA containing supernatant, transfer to a new tube and **save**.
18) (**Optional**) Repeated elution can yield additional 10-20% of DNA.

Keep the cfDNA containing supernatant at +4°C for next-day use or at -20°C for long-term storage.

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DNA isolation protocol from large volumes of urine:

1) Collect urine in 15 ml falcon tubes
2) Add **20 µl Urine Stabilization Solution per 10 ml** of urine
3) Centrifuge 15 min at 3500 rpm (~2400 g)
4) Transfer supernatant to fresh tubes
5) Add **2 ml SubX™**, per 10 ml urine, mix and incubate 5-10 min at room temperature
6) Add **Binding Matrix** (**60 µl**), mix and incubate 5-10 min at room temperature
7) Centrifuge 30 min at >3500 rpm (>2400 g)
8) Discard supernatant, invert tube onto paper towel and let remaining liquid to drain
9) Resuspend [**SubX-DNA-BM**] pellet in **0.5 ml Wash-1** solution, mix and transfer into 1.5 ml Eppendorf tube, incubate 3-4 min
10) Centrifuge for 1 min at 14000 rpm
11) Discard supernatant by aspirating (not to disturb the pellet!)
12) Resuspend pellet in **0.5 ml Wash-G**, mix and incubate 3-4 min
13) Centrifuge for 1 min at 14000 rpm
14) Discard supernatant
15) Resuspend pellet in **1 ml Wash-2**, mix and incubate 3-4 min
16) Centrifuge for 1 min at 14000 rpm
17) Discard supernatant
18) Resuspend pellet in **1 ml Wash-2**, mix and incubate 3-4 min
19) Centrifuge for 1 min at 14000 rpm
20) Discard supernatant
21) Centrifuge 15 sec at 14000 rpm
22) Aspirate leftover using 10-20 µl tip
23) Resuspend pellet in **50 – 200 µl** EB (elution buffer)
24) Elution: Incubate at 60^{0}C for 5 minutes at 1200 rpm on Thermomixer
25) Centrifuge at 14000 rpm for 1 min
26) (**Optional**) Repeated elution can yield additional 10-20% of DNA.

Keep the cfDNA containing supernatant at +4°C for next-day use or at -20°C for long-term storage.