SubX® Plasma cfDNA Isolation Kit

INSTRUCTION MANUAL

Catalog No. CFDNA-0050
Up to 50 isolations

For Research Use Only

Product **Description**

SubX® kit is designed for isolation of 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. 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. The specially designed solid-phase matrix does not require addition of (unwanted) exogenous RNA to prevent non-specific DNA adsorption.

DNA-binding SubX® matrix allows:

- Scaling up procedure to 50 ml of starting material or scaling down to extra low volumes and adaptation of the protocol for 96/384-wells format;
- Separation of cfDNA from the bulk of proteins in a single vortex-spin step without employing chaotropic agents thus speeding-up isolation procedure and reducing the costs;
- Efficiently bind cell-free DNA of virtually any size ranging from 100 Kbp down to 50 bp therefore extending downstream applications.

Kit components, shipping and storage conditions:

- **SubX® Solution** (3 x 0.5 ml). Ready-to-use. **Store refrigerated +4°C.**
- **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-2 Solution** (10 ml). **Add 50 ml of 96% molecular grade ethanol before use.** Store at room temperature in a dark place.

DNA Isolation from 0.2 ml plasma/serum

(50 isolations)

1) Add **30 µl** of **SubX®** solution to **0.2 ml** of human plasma, close the tube and immediately vortex for 10-15 seconds. Turbidity occurs. Incubate 3 min at room temperature; vortex 3-4 times during incubation.
2) Vortex **Binding Matrix** for 15 sec; open the tube and add **10 µl** of **Binding Matrix** slurry to **SubX®**-plasma mixture, close the tube and incubate 3 min at room temperature; briefly (~10 sec) vortex 3-4 times during incubation.
3) 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.
4) Add **0.3 ml** **Wash-1** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;
5) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
6) Add **0.4 ml** **Wash-2** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;
7) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
8) Add **0.4 ml** **Wash-2** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;
9) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
10) Add **0.4 ml** **Wash-2** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;
11) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
12) Obligatory 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.
13) Add 20-50 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.

DNA Isolation from 0.5 ml plasma/serum

(20 isolations)

1) Add **70 µl** of **SubX®** solution to **0.5 ml** of human plasma, close the tube and immediately vortex for 10-15 seconds. Turbidity occurs. Incubate 3 min at room temperature; vortex 3-4 times during incubation.
2) Vortex **Binding Matrix** for 15 sec; open the tube and add **15 µl** of **Binding Matrix** slurry to **SubX®**-plasma mixture, close the tube and incubate 3 min at room temperature; briefly (~10 sec) vortex 3-4 times during incubation.
3) 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.
4) Add **0.4 ml** **Wash-1** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;
5) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
6) Add **0.5 ml** **Wash-2** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;

7) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
8) Add **0.5 ml** **Wash-2** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;
9) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
10) Add **0.5 ml** **Wash-2** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;
11) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
12) Obligatory 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.
13) Add 20-50 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.

DNA Isolation from 1 ml plasma/serum.

(10 isolations)

1) Add **140 µl** of **SubX®** solution to **1 ml** of human plasma, close the tube and immediately vortex for 10-15 seconds. Turbidity occurs. Incubate 3 min at room temperature; vortex 3-4 times during incubation.
2) Vortex **Binding Matrix** for 15 sec; open the tube and add **20 µl** of **Binding Matrix** slurry to **SubX®**-plasma mixture, close the tube and incubate 3 min at room temperature; briefly (~10 sec) vortex 3-4 times during incubation.
3) 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.
4) Add **0.5 ml** **Wash-1** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;
5) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
6) Add **0.6 ml** **Wash-2** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;
7) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
8) Add **0.6 ml** **Wash-2** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;
9) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
10) Add **0.6 ml** **Wash-2** solution to the pellet, close the tube and briefly (~10 sec) vortex 3-4 times for 3 min;
11) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
12) **Obligatory 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.
13) 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.
14) Centrifuge the tube for 1 min at 14,000 rpm, carefully aspirate the cfDNA containing supernatant, transfer to a new tube and **save**.

Important Notes

Read complete protocol before use!

Keep SubX® Solution and Elution Buffer at +4°C, all other components store at room temperature protected from direct sunlight.

For best results, use molecular grade 96% ethanol for preparation of working Wash-2 solution. 100% ethanol (200-proof) is less optimal.

To prevent contamination of circulating cell free DNA with cellular DNA centrifuge plasma for at least 1-2 min at 14000 rpm before use and carefully aspirate supernatant taking care not to disturb the pellet. Transfer into a new tube.

We recommend using Posi-Click 1.5 ml Eppendorf tubes to prevent material loss during vortexing due to possible cap opening.

The Binding Matrix can be separated from the liquid phase with magnetic stands such as DynaMag™-2 Magnet. If using a magnetic separation protocol, centrifugation for a short time is necessary to collect all materials from the tube cap.

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