Capital Biosciences
innovative solutions for life sciences

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SubX™ Cell Culture Media cfDNA Isolation Kit

INSTRUCTION MANUAL (v1.2)

Catalog No. CMDNA-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 cell culture medium 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 (unwanted) 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:

- **SubX™ Solution** (2 x 7 ml). Ready-to-use. Store at room temperature.
- **Condition Solution** (1.5 ml) Ready-to-use. 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** (11 ml). **Add 55 ml of 96% molecular grade ethanol before use.** Store at room temperature in a dark place.
- **Elution Buffer**: (2x1.5 ml); Ready-to-use.

Kits are shipped at ambient temperatures.

Important Notes

- To prevent contamination of circulating cell free DNA with cellular DNA centrifuge medium 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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Protocol:
DNA Isolation from 1 ml or 2 ml medium.
(50 isolations)

1) Add **30 µl Condition Solution** to 1 ml medium or **60 µl** to **2 ml** and vortex for 5 seconds.
2) Add **140 µl** of **SubX™**, 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** for 15 sec; open the tube and add **20 µl** of **Binding Matrix** slurry to **SubXTM**-medium 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.
19) 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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Protocol:

DNA Isolation from 5 ml or larger volumes of medium.
(up to 10 isolations)

1) Add 150 µl Condition Solution per 5 ml medium in 40 ml Nalgene centrifugation tube and vortex for 5 seconds.
2) Add **700 µl** of **SubX™** solution, close the tube and briefly (~10 sec) and immediately vortex. Turbidity may occur, vortex mixture and incubate 3 min at room temperature; vortex 3-4 times during incubation.
3) Vortex **Binding Matrix** for 15 sec; open the tube and add **30 µl** of **Binding Matrix** slurry to [**SubX™+medium**] mixture, close the tube and incubate 5 min at room temperature; briefly (~10 sec) vortex 3-4 times during incubation.
4) Centrifuge the tube for 10 min at 10000 rpm in high-speed centrifuge, aspirate the supernatant carefully by pipetting taking care not to disturb the **Binding Matrix**, discard supernatant.
5) Add 1.0 ml **Wash-1** solution to the pellet, mix and transfer suspension to 1.5 ml tube, 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 1.0 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 another 1.0 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) **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.
14) Add 50-100 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 or employ thermo-shaker.
15) Centrifuge the tube for 1 min at 14,000 rpm, carefully aspirate the cfDNA containing supernatant, transfer to a new tube and ***save***.

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