Capital Biosciences
innovative solutions for life sciences

DNA Isolation from 1 ml or 2 ml plasma/serum.
(50 isolations)

1) Add **30 µl 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 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™**-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 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) **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 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.
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) (**Optional**) Repeated elution can yield additional 10-20% of DNA.
17) 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.

Contact Us

Capital Biosciences, Inc.
900 Clopper Rd, Suite120
Gaithersburg, MD 20878
Phone: **1-800-475-2812**
Email: **info@capitalbiosciences.com**
Web: www.capitalbiosciences.com

Capital Biosciences
innovative solutions for life sciences

SubX™ Plasma cfDNA isolation kit

INSTRUCTION MANUAL (v1.5)

Catalog No. CFDNA-0050
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. 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** (2x7 ml). Ready-to-use. **Store refrigerated +4°C.**
- **Plasma 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 (12 ml)**. Add 18 ml of 96% molecular grade ethanol before use.**
- **Wash-2 Solution** (2x11 ml). **Add 55 ml of 96% molecular grade ethanol before use.**
  Store at room temperature.
- **Elution Buffer**: (5 ml); Ready-to-use. **Store refrigerated +4°C.**

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.
- 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.

Capital Biosciences
innovative solutions for life sciences

DNA Isolation from 0.2 ml plasma/serum
(100 isolations)

1) Add **6 µl Plasma Condition Solution** and vortex for 5 seconds.
2) 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.
3) 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.
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 **0.3 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 **0.3 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 **0.4 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 **0.4 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 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.
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) (**Optional**) Repeated elution can yield additional 10-20% of DNA.
17) Keep the cfDNA containing supernatant at +4°C for next-day use or at -20°C for long-term storage.

Capital Biosciences
innovative solutions for life sciences

DNA Isolation from 0.5 ml plasma/serum
(65 isolations)

1) Add **15 µl Plasma Condition Solution** and vortex for 5 seconds.
2) 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.
3) 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.
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 **0.4 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 **0.4 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 **0.5 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 **0.5 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 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.
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) (**Optional**) Repeated elution can yield additional 10-20% of DNA.
17) Keep the cfDNA containing supernatant at +4°C for next-day use or at -20°C for long-term storage.