Capital Biosciences
innovative solutions for life sciences

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SubX™ RNA Isolation Kit

INSTRUCTION MANUAL (v1.0)

Catalog No. VIR-0100
Up to 100 isolations.

For Research Use Only

Product Description

SubX™ kit is designed for isolation of circulating cell-free RNA (cfRNA) and viral RNA directly from the medium. Our technology is based on the use of proprietary bi-functional substance (SubX™) that binds DNA and RNA under physiological conditions (e.g. directly in biological liquids), followed by adsorption on a solid phase matrix. Since SubX™ captures DNA and RNA via phosphate residues (groups) it allows for elimination of bias related to both AT/CG content and fragments length, thus improving extraction efficacy and accuracy of downstream applications.

Kit components, shipping and storage conditions:

- **SubX™ Solution** (2 x 7 ml). Ready-to-use. **Store refrigerated +4°C.**
- **Binding Matrix** (2 x1 ml). Ready-to-use. Store at room temperature in a dark place.
- **Wash-1 Solution** (60 ml). Ready-to-use. Store at room temperature in a dark place.
- **Wash-2 Solution** (2 x 11 ml). **Add 55 ml of 96% molecular grade ethanol before use.** Store at room temperature in a dark place.
- **Elution Buffer**: (5 ml); Ready-to-use. **Store refrigerated +4°C.**

Kits are shipped at ambient temperatures.

Important Notes

- 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:
RNA Isolation from 1 ml or 2 ml medium (cell culture, VTM, etc).
(100 isolations)

1) Add **100 µl** of **SubX™**, to 1 ml VTM, close the tube and immediately vortex for 10-15 seconds. Turbidity may occur. Incubate 10-15 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™**-medium mixture, close the tube and incubate 5-10 min at room temperature; briefly (~10 sec) vortex 3-4 times during incubation.
3) 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.
4) Add **0.4 ml Wash-1** solution to the pellet, close the tube and briefly (~10 sec) vortex and incubate for 5 min;
5) Add **0.2 ml 96% Ethanol**, close the tube and briefly (~10 sec) vortex and incubate for 5 min.
6) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
7) Add **0.6 ml Wash-2** solution to the pellet, close the tube and briefly (~10 sec) vortex and incubate for 5 min
8) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
9) Repeat steps 7 and 8 (once or twice)
10) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
11) **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.
12) Add 30-50 µl of **Elution** Buffer to the pellet, tightly close the tube and incubate at +50°C for 5 min. Briefly (~10 sec) vortex the tube 5-6 times during incubation.
13) Centrifuge the tube for 1 min at 14,000 rpm, carefully aspirate the RNA containing supernatant, transfer to a new tube and **save**.
14) (**Optional**) Repeated elution can yield additional 10-20% of RNA.
15) Keep the RNA containing supernatant at -20°C or -80°C for long-term storage.

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Protocol:

RNA Isolation from saliva.

1) Centrifuge crude saliva to pellet solid food remains
2) Aspirate carefully supernatant not to disturb the pellet and transfer to new tube.
3) Add equal volume of PBS (e.g. 0.5 ml saliva + 0.5 ml PBS)
4) Add **140 µl** of **SubX™** solution, close the tube and briefly (~10 sec) and immediately vortex. Turbidity may occur, vortex mixture and incubate 5 min at room temperature; vortex 3-4 times during incubation.
5) Optional: sample can be stored at this point for at least 72 hours at room temperature before processing further, if necessary (lack of space or time).
6) Vortex **Binding Matrix** for 15 sec; open the tube and add **20 µl** of **Binding Matrix** slurry to **SubX™**-medium mixture, close the tube and incubate 5-10 min at room temperature; briefly (~10 sec) vortex 3-4 times during incubation.
7) 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.
8) Add **0.4 ml Wash-1** solution to the pellet, close the tube and briefly (~10 sec) vortex and incubate for 5 min;
9) Add **0.2 ml 96% Ethanol**, close the tube and briefly (~10 sec) vortex and incubate for 5 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 and incubate for 5 min
12) Centrifuge the tube for 1 min at 14,000 rpm, aspirate and discard supernatant;
13) Repeat steps 7 and 8 (once or twice)
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** Buffer to the pellet, tightly close the tube and incubate at +50°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 RNA containing supernatant, transfer to a new tube and **save**.
18) (**Optional**) Repeated elution can yield additional 10-20% of RNA.
19) Keep the RNA containing supernatant at -20°C or -80°C for long-term storage.