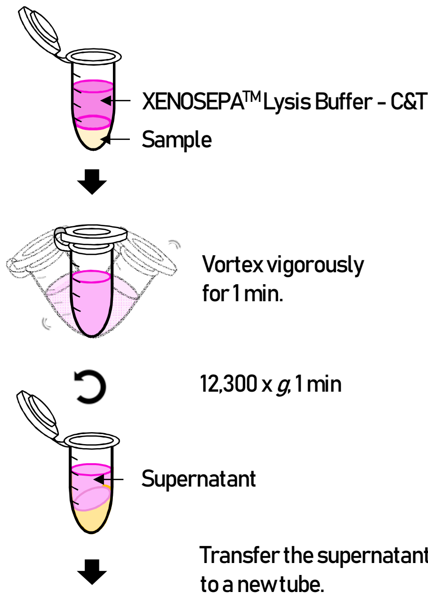
Quick Guide

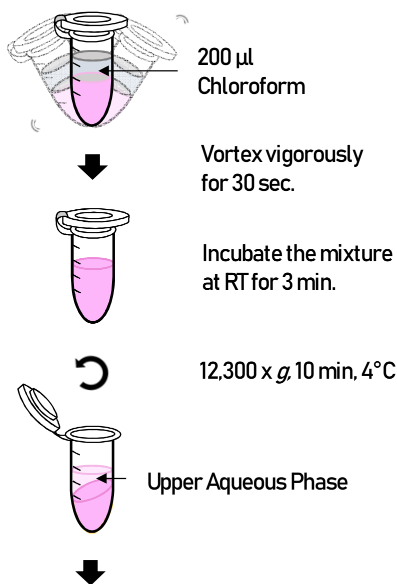
Step 1 ~ 3

Lyse the sample in XENOSEPA™ Lysis Buffer – C&T.



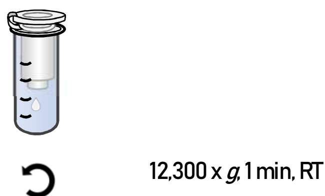
Step 4 ~ 5

Add 200 ul of chloroform and perform phase separation..



Step 6

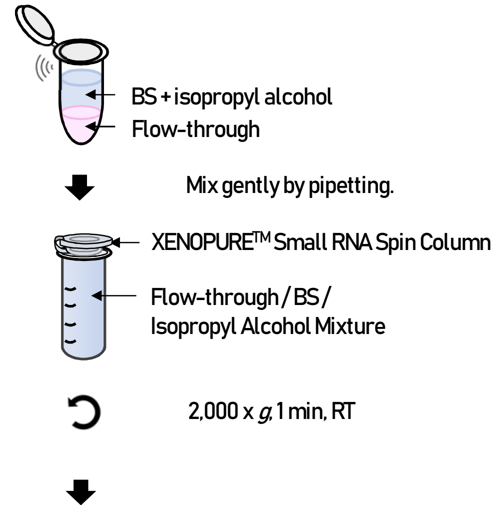
Transfer the upper aqueous phase into gDNA removal column.



<u>Collect the flow-through</u>

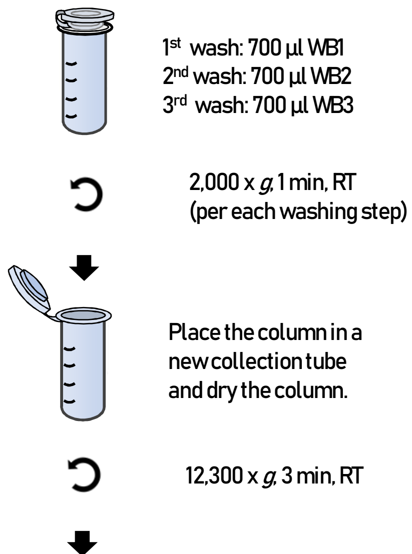
Step 7 ~ 10

Mix BS and isopropyl alcohol with the flow-through and load the mixture onto the RNA column.



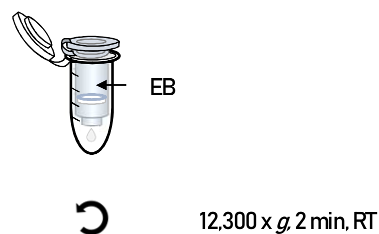
Step 11 ~ 14

Discard the flow-through and wash the column.



Step 15

Transfer the column into a new microcentrifuge tube and elute RNA.



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Protocol: XENOPURE™ C&T Small RNA Purification Kit (Mammalian cells and tissues)

1. Lyse and homogenize samples in 700 µl XENOSEPA™-C&T Lysis Buffer.

- for mammalian tissues Homogenize 20 ~ 100 mg of tissue sample in 700 µl XENOSEPA™-C&T Lysis Buffer using homogenizer. - for mammalian cells Harvest 10^5 ~ 10^7 cultured cells in 1.5 ml microcentrifuge tube and add 700 µl of XENOSEPA™-C&T Lysis Buffer.

2. Vigorously vortex the mixture for 1 min.
3. Centrifuge the mixture for 1 min at 12,300 x *g* and carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube.
4. Add 200 µl of chloroform and vortex for 30 sec.
5. Incubate the mixture at room temperature (15–25°C) for 3 min and centrifuge the mixture for 10 min at 12,300 x *g* at 4°C.
6. Transfer the upper aqueous phase to a genomic DNA removal column and centrifuge for 1 min at 12,300 x *g* at room temperature (caution: Avoid transferring any interphase). Discard the removal column and **collect the flow-through** in a new 1.5 ml microcentrifuge tube.
7. Mix the flow-through with 0.5 volume of Binding Solution (BS) gently (e.g., for 300 µl of aqueous phase, add 150 µl of BS).
8. Add an equal volume of isopropyl alcohol and mix gently (e.g., for 450 µl mixture, add 450 µl isopropyl alcohol).
9. Pipet up to 700 µl of the mixture, including any precipitate, into a XENOPURE™ Small RNA Spin column in a 2 ml collection tube. Close the lid and centrifuge at 2,000 x *g* for 1 min at room temperature. Discard the flow-through.
10. Repeat step 9 using the remainder of the sample.
11. Add 700 µl Washing Buffer (WB 1) to the XENOPURE™ Small RNA Spin column. Close the lid, and centrifuge for 1 min at 2,000 x *g*. Discard the flow-through.
12. Add 700 µl Washing buffer (WB 2) to the XENOPURE™ Small RNA Spin column. Close the lid, and centrifuge for 1 min at 2,000 x *g*. Discard the flow-through.
13. Add 700 µl Washing buffer (WB 3) to the XENOPURE™ Small RNA Spin column. Close the lid, and centrifuge for 1 min at 2,000 x *g*. Discard the flow-through.
14. Place the XENOPURE™ Small RNA Spin column in a new 2 ml collection tube (supplied). Open the lid of the spin column and centrifuge at 12,300 x *g* for 3 min to dry the membrane. Discard the flow-through and the collection tube.
15. Place the XENOPURE™ Small RNA Spin column in a new 1.5 ml microcentrifuge tube. Add 40 µl of Elution Buffer (EB) directly to the center of the XENOPURE™ Small RNA Spin column membrane. Close the lid, and centrifuge for 2 min at 12,300 x *g* to elute the RNA.

※ XENOPURE RNA Purification Kit has been designed to effectively eliminate contaminants such as genomic DNA, proteins, etc. during the RNA purification process. This may result in lower RNA quantitative values than those of other company's.

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