SMARTPURE Protocol

Total RNA

SK-TOPU-100

Please work in an RNase free area and use RNase free tubes and buffers

Sample Preparation

1. Add 175 µL of **Lysis Buffer** to ≤ 30 mg of sample in a 1.5 or 2.0 mL centrifuge tube and mix well.

→ For liquid sample use 75 µL of Lysis Buffer / 100 µL sample.

Protein Removal

2. Add 350 µL of **RD Buffer**, mix well and incubate at 70°C for 4 min.

→ Please increase incubation time for hard sample.

3. Centrifuge at 12 000 × g for 10 min and transfer the supernatant into a clean tube.

RNA Binding

4. Add 200 µL of **ethanol**, mix well and transfer the mixture to a SmartPure column previously placed into a collection tube (2mL).
5. Centrifuge at 12 000 × g for 60 sec.
6. Add 600 µL of **Wash Buffer** to the SmartPure column, centrifuge at 12 000 × g for 30 sec and discard the flow-through.

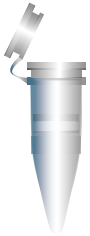
DNA Removal

7. Prepare a mixture containing 40 µL of **DNase Buffer**, 9 µL of **$MnCl_2$** and 1 µL of **DNase I**. Add it to the SmartPure Column. Incubate at room temperature for 15 min.
8. Add 200 µL of **DNase Stop Buffer** to the SmartPure column, centrifuge at 12 000 × g for 30 sec and discard the flow-through.

Washing

9. Add 600 µL of **Wash Buffer** to the SmartPure column, centrifuge at 12 000 × g for 30 sec and discard the flow-through.
10. Add 250 µL of **Wash Buffer** to the SmartPure column, centrifuge at 12 000 × g for 60~120 sec and discard the flow-through.

Total RNA Recovery



11. Transfer the SmartPure column to an RNase free clean 1.5 mL centrifuge tube.
12. Add 50~100 µL of **Elution Buffer** (or RNase-free water pH>7.0) to the SmartPure column and incubate at room temperature for 1 min.
13. Centrifuge at 12 000 × g for 60 sec to recover the total RNA in the microcentrifuge tube.

→ The RNA can be used directly or stored at -80°C for further usage.

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The SmartPure Total RNA kit ensures an easy, fast and effective purification of total RNA from animal tissues, cells, bacteria and other species (Plant tissues are not recommended).

FIRST USE

- Add required ethanol to the Wash and DNase Stop buffers (see labels on the bottles) and mix well.

Kit components (100 preps)

Component	Amount
SmartPure Lysis Buffer	17.5 mL
SmartPure RD Buffer	35 mL
SmartPure DNase Buffer	4 mL
SmartPure $MnCl_2$	900 µL
SmartPure DNase I	100 µL
SmartPure DNase Stop Buffer	10 mL
SmartPure Wash Buffer	2 x 32 mL
SmartPure Elution Buffer	20 mL
SmartPure Columns	100

Material not supplied

- Sterile 1.5 mL microcentrifuge tubes
- 10/100/1000 µL tips
- Microcentrifuge (14 000 x g)
- Vortex mixer
- Absolute ethanol
- ß-mercaptoethanol

Shipping & Storage conditions

The SmartPure Total RNA Kit is shipped at room temperature. The kit is stable for 18 months if stored at room temperature (15-25°C).

The DNase I should be stored at -20°C.

Troubleshooting

Low RNA recovery

- RNA may be impaired in rich oxidase content samples therefore reducing recovery yield. Adding 20 µL of ß-mercaptoethanol per mL of Lysis buffer should prevent RNA alteration. Lysis buffer containing ß-mercaptoethanol must be stored at 4°C.
- Please wear gloves, use RNase free clean tips and tubes and work in an RNase free area to avoid any RNase contamination.
- Please store RNA at -80°C and avoid long term storage.

Presence of DNA

Please note that the DNAse mixture containing DNase buffer, $MnCl_2$ and DNase I must be fresh prepared and not incubated at low temperature. Low temperature may impair DNA digestion.

RNA Analysis

- RNA concentration is calculated from the absorbance value at 260 nm. To assure significance, absorbance readings at 260 nm must be between 0.1 and 1.0.
- An absorbance of 1 unit at 260 nm corresponds to 40 µg of RNA per ml. This relation is only valid for measurements in water. Therefore, any dilution of RNA sample should be done in water. Please use RNase-free cuvettes.
- Concentration of RNA sample = 40 µg/ml x OD260 x dilution factor

Pure RNA has a OD260/OD280 ratio of 1.9-2.1 in 10 mM Tris·Cl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution. If there is contamination with protein or phenol, the OD260/OD280 ratio will be significantly reduced.

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