Tribioscience

Column-Pure RNA Miniprep Kit

Catalog	Unit
TBS6103	50 Preparations

Description

Column-Pure RNA Miniprep Kit is a fast and efficient method for the isolation and purification of total RNA from mammalian cells, tissues, yeast and bacteria. The silica spin column technology allows for rapid recovery of high quality RNA that is ready for downstream applications such as RT-PCR, qPCR, cDNA libraries and Northern blotting.

Component

Product Component	Quantity
Lysis Buffer	50 ml
Wash Buffer 1	50 ml
Wash Buffer 2 (Concentrate)	20 ml
DNase I (RNase-Free)	250 µl
10X DNase I Reaction Buffer	1.0 ml
Nuclease-Free Water	10 ml
RNA Spin Columns & Collection Tubes	50

Store DNase I (RNase-Free) and 10X DNase I Reaction Buffer at -20°C. Store all other components at 18-25°C.

Additional Materials Required (not supplied)

Material	Required For
14.3 M β-mercaptoethanol (β-ME)	All protocols
95-100% Ethanol	All protocols
70% Ethanol	All protocols
Zymolase or Lyticase & Reaction Buffer	Yeast protocol
Lysozyme	Bacteria protocol

Protocol

Perform all centrifugation steps at 12,000 rpm unless stated otherwise.

1. Determine the amount of starting material (do not exceed the following values):

Starting Material	Maximum Amount
Mammalian Cells	1×10^7 cells
Mammalian Tissues	20 mg
Yeast Cells	2×10^7 cells
Bacterial Cells	1×10^9 cells

2. Add 10 µl of β-mercaptoethanol (user supplied) per 1 ml of Lysis Buffer. Mixture is stable for 1 month at room temperature. Add 80 ml of 95-100% Ethanol to Wash Buffer 2 bottle.
3. Harvest cells or tissues.

3a. **Mammalian cells (suspension):** Pellet cells and aspirate media. Proceed to Step 4.

3b. **Mammalian cells (monolayer):** Trypsinize cells, pellet and aspirate media. Proceed to Step 4.

3c. **Mammalian tissue:** Excise tissue sample and determine the weight. Place tissue sample in an appropriate vessel for homogenization. If using a rotor-stator homogenizer: Add 300 µl of Lysis Buffer, and homogenize for 20-40 s. Add 1 volume of 70% Ethanol, pipette to mix. Proceed to Step 5. If using a mortar/pestle and syringe/needle: Immediately add liquid nitrogen and grind sample with mortar and pestle. Transfer tissue powder and liquid nitrogen into a RNase-free 2.0 ml microcentrifuge tube. Open cap to evaporate the liquid nitrogen. Add 300 µl of Lysis Buffer and homogenize using a syringe and 20-gauge RNase-free needle. Repeat passage 5 times. Add 1 volume of 70% Ethanol, pipette to mix. Proceed to Step 5.

3d. **Yeast cells:** Determine the number of cells using a Spectrophotometer. Pellet cells using a 15 ml centrifuge tube at 5000 rpm for 5 min. Discard supernatant and aspirate any excess media. Resuspend the cells in 100 µl of Digestion Mixture (1 M sorbitol, 0.1 M EDTA, pH 7.4 with freshly added 0.1% β-ME and Zymolyase or Lyticase according to supplier's enzyme). Incubate for 10-30 min at 30°C with gentle shaking. Centrifuge at 3000 rpm for 5 min. Discard supernatant and aspirate any excess liquid. Proceed to Step 4.

3e. **Bacterial cells:** Determine the number of cells using a Spectrophotometer. Pellet cells in a 1.5 ml microcentrifuge tube. Discard supernatant and aspirate any excess media. Add 100 µl of Lysozyme (1 mg/ml for gram negative, 2 mg/ml for gram positive) to the pellet, pipette to resuspend. Incubate at room temperature for 5 min. Proceed to Step 4.

4. Add 300 µl of Lysis Buffer (with added β-ME), pipette 10 times to mix, then vortex for 10 s. Add 300 µl of 70% Ethanol, pipette to mix.
5. Apply up to 700 µl of sample into a RNA Spin Column and centrifuge for 30 s. Discard flow-through.
6. Add 350 µl of Wash Buffer 1 to RNA Spin Column and centrifuge for 30 s. Discard flow-through.
7. Prepare DNase I Reaction Mix in a 1.5 ml microcentrifuge tube. For each sample, combine Nuclease-Free Water (43 µl), 10X DNase I Reaction Buffer (5.25 µl) and DNase I (RNase-Free) (4.2 µl). Pipette to mix.
8. Apply 50 µl of DNase I Reaction Mix to the center

of RNA Spin Column. Incubate at room temperature for 15 min.

9. Add 350 µl of Wash Buffer 1 to RNA Spin Column and centrifuge for 30 s. Discard flow-through.
10. Add 500 µl of Wash Buffer 2 (with added ethanol) to RNA Spin Column and centrifuge for 30 s. Discard flow-through and then repeat Step 10.
11. Centrifuge RNA Spin Column empty for 2 min to remove residual ethanol.
12. Transfer RNA Spin Column to a sterile 1.5 ml microcentrifuge tube and discard the Collection Tube. Add 30-50 µl of Nuclease-Free Water to the center of RNA Spin Column. Centrifuge for 1 min. Store purified RNA at -80°C.

For research use only