Column-Pure RNA Miniprep Kit

Cat. No. D518

Store Lysis Buffer, Wash Buffer 1, Wash Buffer 2, and Nuclease-Free H_2O at room temperature. Store 10X DNase I Reaction Buffer, and DNase I (RNase-free) at -20°C.

Product 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.

Product Component	Quantity	Part No.
Lysis Buffer	45.0 ml	P518-1
Wash Buffer 1	45.0 ml	P518-2
Wash Buffer 2 (Concentrate)	20.0 ml	P518-3
DNase I (RNase-free)	250 µl	P518-4
10X DNase I Reaction Buffer	1.0 ml	P111
Nuclease-Free H_2O	5.0 ml	P112
RNA Spin Columns, Collection Tubes	50 preps	P001

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
Zymolyase or Lyticase and Reaction Buffer	Yeast protocol
Lysozyme	Bacteria protocol

Protocol

Perform all protocols with RNase-free plastics in a RNase-free environment. All centrifugation steps are 12,000 rpm at room temperature unless stated otherwise.

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

Starting Material	Maximum Amount
Mammalian cells	1 x 10^7 cells
Mammalian tissues	20 mg fresh or frozen
Yeast	2 x 10^7 cells
Bacteria	1 x 10^9 cells

2. Add 10 µl 14.3 M β-ME per 1 ml Lysis Buffer as needed. 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 tissues:** Excise the 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 seconds. 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:** Determine the number of cells using a Spectrophotometer. Pellet cells using a 15 ml centrifuge tube at 5000 rpm for 5 minutes. 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 minutes at 30°C with gentle shaking. Centrifuge at 3000 rpm for 5 minutes. Discard supernatant and aspirate any excess liquid. Proceed to Step 4.

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3e. **Bacteria:** 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 minutes. Proceed to Step 4.

4. Add 300 µl of Lysis Buffer (with added β-ME), pipette 10 times to mix, then vortex for 10 seconds. Add 300 µl of 70% Ethanol, pipette to mix.

5. Assemble silica Spin Column into a Collection Tube. Apply up to 700 µl mixture to Spin Column and centrifuge for 30 seconds. Discard flow-through.

6. Add 350 µl of Wash Buffer 1 to Spin Column and centrifuge for 30 seconds. Discard flow-through.

7. Prepare DNase I Reaction Mix in a 1.5 ml microcentrifuge tube. For each sample, combine Nuclease-Free H_2O (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 Spin Column. Incubate at room temperature for 15 minutes.

9. Add 350 µl of Wash Buffer 1 to Spin Column and centrifuge 30 seconds. Discard flow-through.

10. Add 500 µl of Wash Buffer 2 (with added ethanol) to Spin Column and centrifuge for 30 seconds. Discard flow-through, and then repeat Step 10.

11. Centrifuge Spin Column for 2 minutes to remove residual ethanol.

12. Transfer Spin Column to a clean 1.5 ml microcentrifuge tube. Add 30 µl of Nuclease-Free H_2O to the center of Spin Column. Centrifuge for 1 minute. Store purified RNA at -80°C.

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