Column-Pure RNA Miniprep Kit

Cat. No. G4002

Store all components at 18-25°C.

Product Description

abm's **Column-Pure RNA Miniprep Kit** is a fast and efficient method for the isolation and purification of total RNA from mammalian cells, animal tissues, plant 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, cDNA libraries and Northern blotting.

Product Component	Quantity
RTL Lysis Buffer	50 ml
RNA Binding Buffer*	15 ml
Buffer RW1	50 ml
Buffer RW2*	20 ml
RNase-Free Water	10 ml
RNA Spin Columns	50
RNA Collection Tubes	50

**Refer to protocol for buffer preparation notes*

Protocol

Perform all centrifugation steps at 12,000 rpm.

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

Starting Material	Maximum Amount
Mammalian Cells	1 x 10^7 cells
Animal Tissue	20 mg
Plant Tissue	150 mg
Yeast Cells	5 x 10^6 cells
Bacterial Cells	1 x 10^8 cells

2. Add 20 µl of 2-mercaptoethanol (user supplied) per 1 ml of RTL Lysis Buffer. Mixture is stable for 2 weeks at room temperature.

3. Harvest cells or tissues.
   3a. **Mammalian cells (suspension)**: Pellet cells and aspirate media. Add 350 µl RTL Lysis Buffer. Pass the lysate five times through a blunt 20-gauge needle fitted to an RNase-Free syringe. Proceed to Step 4.

   3b. **Mammalian cells (monolayer)**: For direct lysis, aspirate media and add 350 µl RTL Lysis Buffer for a 6 mm dish, or 700 µl RTL Lysis Buffer for a 6-10 mm dish. Pass the lysate five times through a blunt 20-gauge needle fitted to an RNase-Free syringe. Proceed to Step 4.

   3c. **Animal tissue**: Homogenize sample using a rotor-stator or mortar and pestle. Transfer powdered tissue into a 1.5 ml microcentrifuge tube. Add 400 µl RTL Lysis Buffer for <10 mg sample, or 700 µl RTL Lysis Buffer for >10 mg sample and mix by vortex. Centrifuge at maximum speed for 3 min and transfer the cleared supernatant into a new 1.5 ml microcentrifuge tube. Proceed to Step 4.

   3d. **Plant tissue**: Homogenize sample using liquid nitrogen and a mortar and pestle. Transfer powdered tissue into a 1.5 ml microcentrifuge tube. Add 700 µl of RTL Lysis Buffer and mix by vortex. Centrifuge at maximum speed for 3 min and transfer the cleared supernatant into a new 1.5 ml microcentrifuge tube. Proceed to Step 4.

   3e. **Yeast cells**: Pellet cells and aspirate media. Add 300 mg of 0.4-0.6g glass beads (user supplied) and 400 µl of RTL Lysis Buffer. Homogenize or vortex for 10 min. Centrifuge at maximum speed for 3 min. Transfer the cleared supernatant into a new 1.5 ml microcentrifuge tube. Proceed to Step 4.

   3f. **Bacterial cells**: Pellet cells and aspirate media. Add 300 mg of 0.4-0.6g glass beads (user supplied) and 400 µl of RTL Lysis Buffer. Homogenize or vortex for 10 min. Centrifuge at maximum speed for 3 min. Transfer the cleared supernatant into a new 1.5 ml microcentrifuge tube. Proceed to Step 4.

   3g. **RNA Clean up**: Adjust the RNA sample to a volume of 100 µl using RNase-Free Water. Add 300 µl of RTL Lysis Buffer and mix. Add 300 µl of 95% Ethanol and mix by vortex. Proceed to Step 5.

4. Add 1 volume of RNA Binding Buffer *(*prior to first time use add 35 ml of 95% Ethanol to bottle)* and mix immediately by pipette.

5. Assemble a Spin Column into a Collection Tube.

6. Add up to 700 µl of sample into the assembled Spin Column. Centrifuge for 1 min and discard the flow-through. Repeat until the entire sample has been transferred.

7. Add 700 µl of Buffer RW1 to Spin Column and centrifuge for 1 min. Discard the flow-through.

8. Add 500 µl Buffer RW2 *(*prior to first time use add 80 ml of 95% Ethanol to bottle)* to Spin Column and centrifuge for 1 min. Discard the flow-through. Repeat Step 8.

9. Centrifuge the Spin Column for 2 min to remove residual wash buffer.

10. Discard the Collection Tube and transfer the Spin Column to a new 1.5 ml microcentrifuge tube. Add 30-50 µl of RNase-Free Water to the center of the Spin Column. Incubate for 2 min at room temperature and centrifuge for 1 min. Store purified RNA at -80°C.

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Troubleshooting

1. Clogged Spin Column
   - There is too much starting material. Reduce and follow amounts outlined in Step 1.
   - Inefficient homogenization of sample. Repeat Step 3 procedures.
2. Poor RNA performance in downstream applications
   - Eluted sample contains a high concentration of salt. Modify the wash step by incubating the column for 5 min at room temperature after adding 500 µl of Buffer RW2, then proceed with centrifugation.
   - Eluted sample contains ethanol. Ensure that Step 9 is followed and the column is dry.
3. RNA is contaminated with DNA
   - Perform optional on-column DNaseI digestion.
   - Incubate sample with Buffer RW1. Repeat procedure from Step 1. At Step 7 add 700 µl Buffer RW1 to the Spin Column and incubate for 5 min before centrifuging. Proceed according to protocol.

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