

# IDPURE™ Spin Column Total RNA MiniPrep Kit

Cat# IDBB2043 100 Preps

## Kit Contents

Components	Size
RLT Solution <sup>a</sup>	70 ml
RW Solution	60 ml
RPE Solution <sup>b</sup>	22 ml
DEPC-Water	5 ml
Spin Column	100
2.0-ml Collection Tube	100

(a) RLT Solution should be kept at 2-8°C after receiving and using. It may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37°C.

(b) Before use, add 88 ml of 100% ethanol to 22 ml RPE Solution. For other volumes of RPE Solution, simply add enough ethanol to make a 4:1 ratio (volume of added ethanol: volume of RPE Solution = 4:1).

Storage: The kit is stable for 12 months at room temperature. For longer storage, keep all contents of the kit cold.

## Principle:

This kit is designed for fast isolation of total RNA from bacteria, yeast, fungi, plant and animal tissues. The kit contains a membrane embedded in a spin column for binding up to 10 µg of RNA. Nucleotides, proteins, salts, and other impurities do not bind to the spin column.

## Applications:

1. Preparation of Total RNA from variable sources.
2. Differential display
3. cDNA synthesis
4. PolyA + RNA selection
5. RNase/S1 nuclease protection

## Features:

1. Preparation of High quality total RNA from animal cells or other sources.
2. Rapid and Economical. Entire procedure takes about 20 minutes (Timing for preparation of samples is not included).
3. High yields
4. No phenol / chloroform extraction, no ethanol precipitation

## Procedure for Isolation of Total RNA from Animal Cells.

1. Sample Preparation:

### A. Harvest cells

(a) Cells grown in suspension: Determine the number of cells. Spin down the appropriate number of cells for 5 minutes at 1,200 rpm in a RNase-free microtube. Carefully remove all supernatant by aspiration, and continue with Step 2 of the protocol

(b) Cells grown in a monolayer. Determine the number of cells. Cells grown in a monolayer in cell-culture vessels can be trypsinized. Aspirate medium, and wash cells with PBS. Aspirate PBS and add 0.1-0.25% trypsin in PBS. After cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer cells to 1.5-ml RNase A-free microtube, spin by centrifugation at 1,200 rpm for 5 minutes. Completely aspirate supernatant, and continue with Step 2 of the protocol.

Note: Incomplete removal of the cell-culture will inhibit lysis and dilute the lysate, affecting the conditions for binding RNA to Spin column. Both effects may reduce RNA yield.

## B. Disrupt cells by addition of RLT Solution

- (a) For pelleted cells loosen cell pellet by flicking the tube and add RLT Solution  
 (b) For monolayer cells. Add RLT Solution to monolayer cells (according to table below). Collect cell lysate with a rubber policeman. Vortex. No cell clumps should be visible before proceeding to Step 3

RLT Solution (μl)	Number of pelleted cells	Dish diameter (cm)
350	$> 5 \times 10^6$	6
600	$> 5 \times 10^6$ to $1 \times 10^7$	6-10

2. Homogenize lysate: Two alternative methods may be used.

- (a) Homogenize for 30 seconds using a rotor-stator homogenizer.  
 (b) Pass lysate at least 5 times through a 20-G(D=0.9mm) needle fitted to a syringe.

3. Add equal volume of 70% ethanol to the homogenized lysate, and mix well by pipetting. Do not centrifuge.

4. Place Spin column to 2.0-ml collection tube. Transfer above ethanol mixture to the column. Spin at 8,000 rpm for 1 minute.

Note: precipitate may form after adding ethanol, but this will not affect the procedure.

5. Discard flow-through. Add 500 μl RW Solution to the column and spin at 8,000 rpm for 1 minute. Discard flow-through and place Spin column back to the same collection tube.

6. Add 500 μl RPE Solution to the column, spin at 8,000 rpm for 1 minute. Discard flow-through and spin once more to remove residue of RPE Solution.

7. Transfer Spin column to a clean RNase-free 1.5 ml microtube. Add 30-50 μl RNase-free water onto the center part of the column; incubate at 50° C for 2 minutes. Spin down at 10,000 rpm for 1 minute. RNA is ready for use or kept at –70°C.

## Procedure for Isolation of Total RNA from Bacteria

1 Samples Preparation: Harvest the appropriate number of cells ( $<1 \times 10^9$ ) by centrifugation at 5000 rpm for 3 minutes at 4°C. Discard supernatant ensuring all media is completely removed.

2. Add 100 μl TE containing lysozyme and incubate. Gram-negative bacteria and Gram-positive bacteria require different amount of lysozyme and incubation periods.

Bacteria	Concentration of Lysozyme in TE	Incubation Time
Gram-negative	400μg / ml	3-5 minutes
Gram-positive	3 mg / ml	5-10 minutes

Invert several times during incubation.

3. Add 350 μl of RLT Solution, mix vigorously. If insoluble material is visible, centrifuge for 2 minutes at 8,000 rpm. Save the supernatant.

4. Add 260 μl of 100% ethanol to the supernatant, mix gently. A precipitate may form by adding ethanol, but it will not affect the procedure.

5. Place a Spin column to 2.0-ml collection tube and transfer the mixture to the column. Spin at 8,000 rpm for 1 minute. Discard flow-through and place the column back to the same collection tube.

6. Add 500 μl of RW Solution to the column, centrifuge at 8,000 rpm for 1 minute, and discard flow-through.

7. Add 500 μl of RPE Solution and spin at 8,000 rpm for 1 minute. Discard the solution in the collection tube and spin once more to remove the residue of RPE Solution.

**Note:** Ensure ethanol is added to RPE Solution before use.

8. Transfer the column to a clean RNase-free 1.5 ml microtube; add 30-50 μl of RNase-free H<sub>2</sub>O onto the center part of the membrane in the column. And incubate at 50 °C for 2 minutes and spin down at 10,000 rpm for 1 minute. Keep RNA sample at –70°C.

## Procedure for Isolation of Total RNA from Tissue, Plant Cells and Filamentous Fungi

1. Sample Preparation: Grind sample under liquid nitrogen to a fine powder using a mortar and pestle. Transfer the mixture of tissue powder and liquid nitrogen to 1.5 ml microtube and allow the liquid nitrogen to evaporate. Do not allow the sample to thaw. Continue immediately with Step 2.

2. Add 450  $\mu$ l of RLT Solution to a maximum of 100 mg of tissue powder and vortex vigorously. Incubation at 50°C may help to disrupt tissue. For samples with high starch content, incubation is avoided to prevent swelling of starch material.
  3. Add 330  $\mu$ l of 100% ethanol to the mixture (step 2). Mix gently.
  4. Place a Spin column onto a 2.0-ml collection tube and transfer the mixture to the column. Spin at 8,000 rpm for 1 minute. Discard flow-through and place the column back to the same collection tube.
  5. Add 500  $\mu$ l of RW Solution to the column, centrifuge at 8,000 rpm for 1 minute, and discard flow-through.
  6. Add 500  $\mu$ l of RPE Solution and spin at 8,000 rpm for 1 minute. Discard the solution in the collection tube and spin once more to remove the residue of RPE Solution.
- Note: Ensure ethanol is added to RPE Solution before use.
7. Transfer the column to a clean RNase-free 1.5 ml microtube, add 30-50  $\mu$ l of RNase-free H<sub>2</sub>O onto the center part of the membrane in the column, and incubate at 50°C for 2 minutes and spin down at 10,000 rpm for 1 minute. Keep RNA sample at -70°C,

### **Procedure for RNA Cleanup**

This kit can also be used to clean up RNA, which is isolated by different methods or after enzymatic reactions.

1. Adjust sample to a volume of 100  $\mu$ l with RNase-free H<sub>2</sub>O, add 350  $\mu$ l of RLT Solution, and mix well. Add 260  $\mu$ l of 100% ethanol, mix gently. A precipitate may form by adding ethanol, but this will not affect the procedure.
2. Place a Spin column in 2-ml collection tube and transfer the mixture solution (Step 1) to the column and spin at 4,000 rpm for 1 minute, discard flow-through.
3. Add 500  $\mu$ l RW Solution to the column and centrifuge at 8,000 rpm for 1 minute. Discard the solution in collection tube.
4. Add 500  $\mu$ l RPE Solution to the column and spin at 8,000 rpm for 1 minute, discard the flow-through and spin once more to completely remove the residue of RPE Solution.
5. Add 30-50  $\mu$ l of RNase-free H<sub>2</sub>O onto the center part of the membrane of the column and centrifuge at 10,000 rpm for 1 minute. Keep RNA sample at -70°C.