Protocol for Total RNA Isolation from Cells

Description

The following protocol is for total RNA isolation from cells using the Total RNA Purification Kit (Catalog #79040). For complete instructions, refer to the Technical Manual (Document #10000005434).

Directions

 Prepare cell lysate from adherent cells or cells in suspension, as indicated below.

Adherent Cells

 Aspirate cell culture medium. Add ice-cold sterile D-PBS as indicated in Table 1. Aspirate D-PBS.

Table 1. Recommended Volumes of PBS, RNA Lysis Buffer + TG, and 100% Isopropanol for Various Cultureware

Cultureware	Volume of D-PBS	Volume of RNA Lysis Buffer + TG	Volume of 100% Isopropanol
T-25 cm² flask	5 mL	500 μL	170 µL
6-well plate	2 mL	250 μL	85 µL
24-well plate	500 μL	100 μL	35 μL
48-well plate	250 μL	100 μL	35 µL
96-well plate	100 μL	100 μL	35 µL

- b) Add RNA Lysis Buffer + TG as indicated in Table 1. Gently rock the plate or flask to completely cover the adherent cells with buffer. Pipette the lysate up and down over the cultureware surface 7 - 10 times.
- c) Collect the lysate and transfer to a new microcentrifuge tube.
- d) Add 100% isopropanol as indicated in Table 1. Mix by vortexing for 5 seconds.
- e) Proceed to step 2 for RNA isolation.

Cells in Suspension

a) In a sterile centrifuge tube, centrifuge cell suspension at 300 x *g* for 5 minutes. Remove and discard supernatant.

- b) Add ice-cold, sterile D-PBS to wash cells. Centrifuge at 300 x *g* for 5 minutes. Remove as much supernatant as possible and discard.
- c) Add RNA Lysis Buffer + TG as indicated in Table 2. Mix well by pipetting up and down 7 10 times, or by vortexing. For $> 2 \times 10^6$ cells, pass the lysate through a 20-gauge needle 4 5 times to shear the genomic DNA.
- Add 100% isopropanol as indicated in Table 2. Mix by vortexing for 5 seconds.
- e) Proceed to step 2 for RNA isolation.

Table 2. Recommended Volumes of RNA Lysis Buffer + TG and Isopropanol per Cell Input Range

Cell Input Range	Volume of RNA Lysis Buffer + TG	Volume of 100% Isopropanol
1 x 10 ² to 5 x 10 ⁵	100 μL	35 μL
$> 5 \times 10^5$ to 2 x 10 ⁶	250 μL	85 μL
> 2 x 10 ⁶ to 5 x 10 ⁶	500 μL	170 μL

- 2. Insert minicolumn into Collection Tube.
- 3. Transfer the lysate to the minicolumn assembly.
- 4. Centrifuge at $12,000 14,000 \times g$ for 30 seconds. Discard the liquid in the Collection Tube and reinsert minicolumn into Collection Tube.
- 5. Add 500 μ L RNA Wash Buffer to the minicolumn. Centrifuge at 12,000 14,000 x g for 30 seconds. Discard the liquid in the Collection Tube and reinsert minicolumn into Collection Tube.
- 6. Prepare DNase I Incubation Mix by combining the reagents as indicated in Table 3, per sample, **in the order listed**.

Table 3. Preparation of DNase I Incubation Mix

Component	Volume Per Sample
DNA Digestion Buffer	24 μL
MnCl ₂	3 μL
DNase I Solution	3 µL



- 7. Mix by gently pipetting up and down; do not vortex. Store on ice.
- 8. Add 30 μ L of fresh DNase I Incubation Mix directly to the minicolumn membrane. Incubate at room temperature (15 25°C) for 15 minutes.
- 9. Add 200 μ L of Column Wash Buffer (with ethanol added) to the minicolumn. Centrifuge at 12,000 14,000 x g for 15 seconds.
- 10. Add 500 μ L of RNA Wash Buffer (with ethanol added) to the minicolumn. Centrifuge at 12,000 14,000 x g for 30 seconds. Remove the minicolumn and transfer to a new Collection Tube. Discard the Collection Tube containing wash buffer.
- 11. Add 300 μ L of RNA Wash Buffer to the minicolumn. Centrifuge at high speed for 2 minutes.
- 12. Transfer minicolumn to an Elution Tube. Add nuclease-free water to the minicolumn membrane as indicated in Table 4. Incubate at room temperature for 1 2 minutes. Centrifuge at $12,000 14,000 \times g$ for 1 minute.
- 13. Remove and discard minicolumn. Store purified RNA at -80°C.

Table 4. Recommended Volume of Nuclease-Free Water per Cell Input Range

Cell Input Range	Volume of Nuclease-Free Water
1 x 10 ² to 5 x 10 ⁵	15 μL
> 5 x 10 ⁵ to 2 x 10 ⁶	30 μL
> 2 x 10 ⁶ to 5 x 10 ⁶	50 μL

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Protocol for Total RNA Isolation



Prepare cell lysate Add 100% isopropanol



Transfer cell lysate to minicolumn assembly



Centrifuge



Add RNA Wash Buffer



Centrifuge



Add DNase I Incubation Mix to minicolumn membrane Incubate 15 minutes

Add Column Wash Buffer



Centrifuge



Add RNA Wash Buffer



Centrifuge



Transfer minicolumn to new Collection Tube Add RNA Wash Buffer



Centrifuge



Transfer minicolumn to Elution Tube Add nuclease-free water Incubate 1 - 2 minutes



Centrifuge



Store purified RNA at -80°C

