TECHNICAL BULLETIN

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

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

Adherent Cells

a) 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 cm^2 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 x 10^6 cells, pass the lysate through a 20-gauge needle 4 - 5 times to shear the genomic DNA.

d) 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 x 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 x *g* for 30 seconds. Discard the liquid in the Collection Tube and reinsert minicolumn into Collection Tube.

5. Add 500 µL 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 µL of fresh DNase I Incubation Mix directly to the minicolumn membrane. Incubate at room temperature (15 - 25°C) for 15 minutes.

9. Add 200 µL of Column Wash Buffer (with ethanol added) to the minicolumn. Centrifuge at 12,000 - 14,000 x *g* for 15 seconds.

10. Add 500 µL 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 µL 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 x *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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