EasyPure® RNA Kit

Please read the manual carefully before use.

Cat. No. ER101

Storage: DNase I and DNase I Reaction Buffer are at -18℃Cor below for one year; others are at room temperature (15℃-30℃) in a dry place for one year.

Description

This kit is designed for the rapid extraction of total RNA from cultured animal cells, tissues and E. coli. The samples are lysed with guanidine isothiocyanate, DNA is digested with DNase I, and RNA is specifically bound to a silica gel membrane spin column. The extracted total RNA is of high purity, free from DNA and protein contamination, and can be used for RT-PCR, qRT-PCR, microarray analysis, Northern blot, and other experiments.

Kit Contents

Component	ER101-01(50 rxns)
Binding Buffer 4 (BB4)	40 ml
Clean Buffer 4 (CB4)	60 ml
Wash Buffer 4 (WB4)	12 ml
Proteinase K (20 mg/ml)	1 ml
DNase I (3 units/μl)	1500 U
DNase I Reaction Buffer	4 ×1 ml
RNase-free Water	10 ml
RNase-free Tube (1.5 ml)	50 each
RNA Spin Columns with Collection Tubes	50 each

Sample Requirements

Sample type	Sample Amount	Amount of BB4 (prepared with 2-mercaptoethanol)
Animal cell	≤5×10^6	0.3-0.6 ml
Animal tissue	≤20 mg	0.3-0.6 ml
Bacterial cell	≤1×10^9	0.35 ml

Sample Handling

A. For Cultured Animal Cells

1. Cell Collection
   - For suspension cells (Do not exceed 5×10^6 cells): Transfer cells to an RNase-free centrifuge tube, centrifuge at 12,000 × g for 5 min at 2-8℃ and then carefully remove all supernatant.
   - For adherent cells (Do not exceed 5×10^6 cells): Treat with trypsin, transfer cells to an RNase-free centrifuge tube and then centrifuge at 12,000 × g for 5 min at 2-8℃ and then carefully remove all supernatant.

   Note: For adherent cells, another method is removing all medium and directly add an appropriate volume of BB4 (add 10 μl β-mercaptoethanol per 1 ml BB4, prepare fresh) to the culture vessel (diameter≤10 cm) and transfer the cell lysate to an RNase-free centrifuge tube, followed by homogenization (step 3 in this part).
2. Lysis

   Gently flick the tube to loosen the cell pellet, add the appropriate volume of lysis buffer BB4 (add 10 μl β-mercaptoethanol per 1 ml BB4, prepare fresh), vortex vigorously until the cell pellet is fully dispersed.

   Note: For ≤1×10^6 cells, use 0.3 ml BB4. For 1×10^6 to 5×10^6 cells, use 0.6 ml BB4.
3. Homogenization

   Mix the solution thoroughly by gently pipetting up and down 5-10 times with RNase-free tips.

4. Centrifugation

Centrifuge at 12,000 × g for 5 min at room temperature. Then transfer the supernatant to a new RNase-free centrifuge tube.

B. Animal Tissue

1. Grinding

Flash-freeze the tissue in liquid nitrogen and grind it into a fine powder using a mortar and pestle. Transfer the powder to a centrifuge tube.

2. Lysis

Add 0.3 ml BB4 (add 10 µl β-mercaptoethanol per 1 ml BB4, prepare fresh) and 15 µl Proteinase K per 10 mg tissue. Mix well and incubate at 56°C for 10-20 min.

Note: For ≤10 mg tissue, use 0.3 ml BB4. For 10-20 mg tissue, use 0.6 ml BB4.

3. Centrifugation

Centrifuge at 12,000 × g for 5 min at room temperature. Transfer the supernatant to a new RNase-free centrifuge tube.

C. Bacterial Cells

1. Cell Collection

Centrifuge the bacterial culture at 12,000 × g for 2 min at 2-8°C (cell number should not exceed 1×10^9). Completely remove all supernatant (incomplete removal may inhibit subsequent cell wall digestion).

2.Resuspension

Resuspend the cell pellet thoroughly in 100 µl TE buffer containing lysozyme (prepare by dissolving 1 mg lysozyme in 100 µl TE buffer).

3.Lysis

Add 350 µl BB4 (add 10 µl β-mercaptoethanol per 1 ml BB4, prepare fresh). Vortex to mix well and incubate at room temperature for 5 min.

4.Homogenization

Mix the solution thoroughly by gently pipetting up and down 5-10 times with RNase-free tips.

5.Centrifugation

Centrifuge at 12,000 × g for 2 min at room temperature. Transfer the supernatant to a new RNase-free centrifuge tube.

RNA isolation

Add 48 ml of absolute ethanol to WB4 before use.

1. For cell and tissue samples: Add equal volume of 70% ethanol to the supernatant. For bacterial samples: Add 250 µl of 100% ethanol to the supernatant and mix well (precipitate may form).

   Note: Prepare 70% ethanol using RNase-free water.

2. Vortex thoroughly to disperse any precipitate. Transfer the entire mixture (including precipitate) to the spin column. Centrifuge at 12,000 × g for 30 sec and discard the flow-through. If the volume exceeds the column capacity, load in multiple steps.
3. Add 500 µl CB4 to the spin column and centrifuge at 12,000 × g for 30 sec. Discard the flow-through.
4. (Optional) For genomic DNA removal: Add 80 µl DNase I working solution [DNase I working solution preparation: Mix 70 µl Reaction Buffer + 10 µl (30 U) DNase I in an RNase-free tube.] directly onto the center of the membrane. Incubate at room temperature for 15 min. Then repeat step 3 once.
5. Add 500 µl WB4 and centrifuge at 12,000 × g for 30 sec. Discard the flow-through.
6. Repeat step 5 once.
7. Centrifuge the empty column at 12,000 × g for 2 min to remove residual ethanol.
8. Transfer the spin column to a new 1.5 ml RNase-free tube. Add 30-100 µl RNase-free water directly onto the center of the membrane. Let it stand at room temperature for 1 min.
9. Centrifuge at 12,000 × g for 2 min to elute the RNA.
10. Store the isolated RNA at -80°C.

Notes

• Ensure that β -mercaptoethanol has been added to BB4.
• Ensure that 96-100% ethanol has been added to WB4.
• All the centrifugation steps are carried out at room temperature.

For research use only, not for clinical diagnosis.

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Service telephone +86-10-57815020
Service email custserv@transgenbiotech.com