EasyPure® RNA Kit

Cat. No. ER101

Storage: DNase I and DNase I Reaction Buffer at -20°C for one year; others at room temperature (15°C-25°C) in a dry place for one year.

Description

EasyPure® RNA Kit provides a simple and fast column based method to isolate total RNA from animal cells, animal tissues, bacteria and yeast. Cells and tissues are enzymatically lysed. DNA is digested with DNase I. RNA is bound to silica membrane. After washing, high quality RNA is eluted from the column. RNA is free of protein contamination, and is suitable for RT-PCR, qRT-PCR and Northern blot.

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 units
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 Requirement

Material	Amount	Volume of BB4/β-ME
Animal cells	$\leq 5\times10^6$	0.3-0.6 ml
Animal tissues	≤20 mg	0.3-0.6 ml
Bacterial cells	$\leq 1\times10^9$	0.35 ml

*BB4 with β-mercaptoethanol: add 10 μl of β-mercaptoethanol for per ml BB4, and the solution must be fresh prepared just before use.

Procedures

Before starting, add 48 ml of 96%-100% ethanol to WB4, mix thoroughly.

Preparing materials

A: Animal cells

(a) Collect cells

For suspension cell ($\leq 5\times10^6$): Transfer cell suspensions to a RNase-free tube and centrifuge at 12,000×g for 5 minutes at 2-8°C then discard the supernatant.

For adherent cell ($\leq 5\times10^6$): Detach the cells by trypsin, transfer cell suspensions to a RNase-free tube and centrifuge at 12,000×g for 5 minutes at 2-8°C, then discard the supernatant. Or remove the upper culture media, and add the appropriate volume of BB4

(b) Cell lysis

Add the appropriate volume of BB4 with β-mercaptoethanol*. Pipette to make cell pellet loose. For cell number$\leq 1\times10^6$, use 0.3 ml of BB4; for cell number between 1×10^6 and 5×10^6, use 0.6 ml of BB4.

(c) Homogenization

Make the cell pellet completely dispersed by vortexing at high speed or pipetting.

(d) Centrifuge at 12,000×g for 5 minutes, transfer cell suspensions to a RNase-free tube.

B. Animal tissues

(a) Grind the sample with liquid nitrogen, and then transfer tissue power to a clean RNase-free tube. Add 0.3 ml of BB4 (BB4 with β-mercaptoethanol*) and 15 μl of Proteinase K for per 10 mg tissue, mix thoroughly by vortexing. Incubate for 10-20 minutes at 56°C.

(b). Centrifuge at 12,000×g for 5 minutes at room temperature and transfer the supernatant to a clean RNase-free tube.

C. Bacterial cells

(a). Centrifuge bacterial culture at 12,000×g for 2 minutes at 2-8°C to pellet bacterial cells(≤1×10^9). Discard the supernatant.
Note: If the supernatant is not completely removed, it may inhibit the cell wall digestion in the second step.

(b). Add 100 μl of TE/lysozyme buffer (add 1 mg lysozyme to 100 μl of TE buffer) to the pellet.Vortex at high speed to resuspend the pellet completely.

(c). Add 350 μl of BB4 with β -mercaptoethanol* and mix thoroughly by vortexing. Incubate at room temperature for 5 minutes.

(d). Pipette up and down for 5-10 times with RNase-free tip to homogenize the solution.

(e). Centrifuge at 12,000×g for 5 minutes at room temperature and transfer the supernatant to a clean RNase-free tube.

RNA Purification

All following centrifugation steps are carried out at room temperature.

(a) For animal cell and tissue: Add equal volume of 70% ethanol to the lysate (use RNase-free Water to prepare for 70% ethanol).
For bacterial cell: Add 250 μl of 96-100% ethanol to the lysa.te

(b) Vortex thoroughly to disperse the precipitate which may form after adding ethanol. Centrifuge briefly and add all the lysate into the spin columns, centrifuge at 12,000×g for 30 seconds, discard the flow through (if the volume of lysate is larger than the spin column can hold, repeat the step).

(c) Add 500 μl of CB4 to the spin column. Centrifuge at 12,000×g for 30 seconds. Discard the flow through.
Optional: When genomic DNA-free RNA is required, add 80 μl of DNase I working solution (the working solution is prepared by mixing 10 μl of DNase I and 70 μl of reaction buffer) and incubate for 15 minutes at room temperature.

(d) Repeat step (c) once.

(e) Add 500 μl of WB4 (check to make sure that ethanol has been added) into the spin column. Centrifuge at 12,000×g for 30 seconds at room temperature. Discard the f low through.

(f) Repeat step (e) once.

(g) Centrifuge the empty column at maximum speed (≥12,000 ×g) for 2 minutes at room temperature in order to remove ethanol residue and then air-dry the column matrix for several minutes.

(h) Place the spin column into a clean 1.5 ml RNase free tube. Add 30-100 μl of RNase-free Water into the spin column matrix and incubate at room temperature for 1 minute.

(i) Centrifuge at 12,000×g for 2 minutes to elute RNA.

(j) 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 toWB4.
- All the centrifugation steps are carried out at room temperature.

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