EasyPure® Blood RNA Kit

Cat. No. ER401

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

Description

EasyPure® Blood RNA Kit provides a simple and fast column based method to isolate total RNA from 50 µl-1.5 ml of fresh or anticoagulated blood. Blood is lysed and DNA is digested with DNase I. RNA is bound to silica membrane. After washing, high quality RNA is eluted. Purified RNA is suitable for RT-PCR, qRT-PCR and Northern blot.

Kit Contents

Component	ER401-01 (50 rxns)
Red Cell Lysis Buffer 2 (RCL2)	125 ml
Binding Buffer 7 (BB7)	40 ml
Clean Buffer 7 (CB7)	60 ml
Wash Buffer 7 (WB7)	12 ml
DNase I (3 units/µl)	1500 units
DNase I Reaction Buffer	4×1 ml
RNase-free Water	10 ml
RNA Spin Columns with Collection Tubes	50 each
RNase-free Tube (1.5 ml)	50 each

Sample Requirement

Fresh or anticoagulated blood can be kept at 2-8°C for one week. Do not freeze blood sample. Blood sample should be extracted as soon as possible and mixed well before use.

Amount of Blood	Volume of BB7
<500 µl	300 µl
500 µl-1.5 ml	600 µl

Procedure

Before starting, add 48 ml 96-100% ethanol to WB7, mix thoroughly.

1. Add 2.5 volume of RCL2 to the 1 volume of blood, mix thoroughly by inverting 4-6 times. Incubate on ice for 10 minutes, mix 2-3 times by inverting during the incubation.
2. Centrifuge the lysate at 400×g for 10 minutes at 2-8°C, discard the supernatant.
   Optional: If the obtained cell pellet is very red, add 500 µl of RCL2 to resuspend the cell pellet. And then centrifuge at 400×g for 10 minutes at 2-8°C, discard the supernatant.
3. Add the appropriate volume of BB7 according to the sample requirement, mix thoroughly to disperse the cell pellet by vortexing.
   For: ≤ 500 µl normal human blood or ≤ 3×10^6 white blood cells, use 300 µl of BB7
   For: 500 µl-1.5 ml normal human blood or 3×10^6-1×10^7 white blood cells, use 600 µl of BB7
4. Add 0.5 volume of 96-100% ethanol to 1 volume of BB7 to the lysate (e.g. if 300 µl of BB7 used in step 3, add 150 µl of 96-100% ethanol to the lysate in this step). Mix thoroughly by vortexing to disperse the precipitation which may form after addition of ethanol. All following centrifugation steps are performed at room temperature.
5. Centrifuge briefly and add the entire contents to a spin column. Centrifuge at 12,000×g for 1 minute, discard flow-through.

6. Add 500 µl of CB7 to the spin column, centrifuge at 12,000×g for 30 seconds, discard flow-through.
   Optional: When genomic DNA-free RNA is needed, add 80 µl of DNase I working solution (The working solution is prepared by mixing by 10 µl of DNase I with 70 µl of DNase I Reaction Buffer) and incubate at room temperature for 5 minutes.
7. Repeat step 6 once without DNase digestion.
8. Add 500 µl of WB7(check to make sure that ethanol has been added) to the spin column, centrifuge at 12,000×g for 30 seconds, discard flow-through.
9. Repeat step 8 once.
10. Centrifuge the empty column at maximum speed (≥12,000×g) to remove ethanol residue, and then air-dry the column matrix for several minutes.
11. Place the spin column into a clean 1.5 ml of RNase-free tube, add 30-100 µl of RNase-free Water to the center of the column matrix, and incubate at room temperature for 1 minute.
12. Centrifuge 12,000×g for 2 minutes to elute RNA.
13. Store the eluted RNA at -80°C.

Notes

Use unfrozen fresh blood treated with anticoagulant for this kit.

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