

# Data sheet

## Blood/Cultured Cell Total RNA Kit

Cat. No: AN0145 (50 reactions)

Cat. No: AN0146 (100 reactions)

### Description

**Blood/Cultured Cell Total RNA Kit** is a simple and rapid method for high-quality total RNA purification from **whole blood and cell culture**.

The kit is based in RNA ability to bind silica in the presence of high concentrations of chaotropic salts. This method first lyses cells, binds RNA to silica-based membranes, washes RNA with ethanol-contained wash buffer and then elutes purified RNA by RNase-free ddH<sub>2</sub>O. It takes 30 min for an entire procedure, and the purified RNA is ready for RT-PCR, northern blotting, primer extension and cDNA library construction.

### Features

- **High yields:** 2-30 µg; depends on type of sample.
- **Ready to use** RNA.
- **Just a few minutes** procedure (about 30 min).
- **Mini format**

### Quality Certifications

Total RNA is isolated from a 300 µl of fresh whole human blood using the **Blood/Cultured Cell Total RNA Kit**. Purified RNA is quantified using a spectrophotometer with a typical yield of 2-3 µg of total RNA and A260nm/A280nm ratio of 1.8-2. Quality is further checked by agarose gel electrophoresis.

Kit Components Item	(Reactions)	
	50	100
RBC Buffer	120 ml	240
Buffer BLY*	25 ml	45 ml
Wash Buffer 1 (WB1)	30 ml	60 ml
Wash Buffer 2 ** (WB2)	15 ml	30 ml
RNase-free ddH <sub>2</sub> O	10 ml	10 ml
RNAprep spin column	50	100
Filter Column	50	100
Collection tube (2mL)	100	200
1.5 ml microtube	50	100

#### Note

\*Before beginning, prepare a fresh amount of Buffer BLY containing 1% 2-mercaptoethanol (β-ME) [Not included] for each purification procedure. Add 10 µL β-ME for each 1 mL Lysis Buffer

\*\*Add the volume ethanol (96%-100%) specified [Not included] to WB2 Buffer prior to initial use (see bottle label for volume). After ethanol has been added, mark the bottle to indicate that this step has been completed.

#### Kit Storage:

**Blood/Cultured Cell Total RNA Kit** can be stored at room temperature. The kit components are stable for 1 year, if stored properly.



Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water.

β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing.

*(Continued on reverse side)*

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## DETAILED PROTOCOL

### For Human Whole Blood

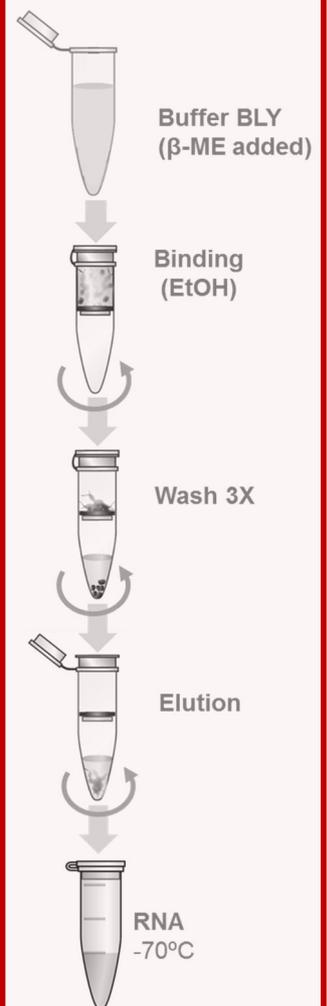
1. Collect fresh human blood in an anticoagulant-treat collection tube.
2. Add 200-300µl human whole blood to an appropriately centrifuge tube (1.5 ml). (Not provided)
3. Mix 5 volume of RBC Buffer with 1 volume of the sample and mix well by inversion.
4. Incubate on ice for 10 min. Vortex briefly 2 times during incubation.
5. Centrifuge for 1 min at 3000g to form a cell pellet and discard the supernatant completely.
6. Add 600 µl of RBC Buffer to resuspend the cell pellet by briefly vortexing.
7. Centrifuge for 1min at 3000g to form a cell pellet again and discard the supernatant completely.
8. Follow the **General Protocol**

### For Cell culture

1. Pellet  $1-5 \times 10^7$  cells by centrifuge at 3000g for 5 min and remove all the supernatant.
2. Follow the **General Protocol**

### General Protocol

1. Add 350µl of **Buffer BLY** (β-ME added) to the cell pellet and vortex vigorously. In order to release all RNA in the sample, it is required to disrupt the sample completely.
2. Place a **Filter Column** in a 2 ml **Collection tube** and transfer the sample mixture to the filter column. Centrifuge at full speed for 2 minutes.
3. Carefully transfer the clarified filtrate to a new 1.5 ml microcentrifuge tube.
4. Add 1 volume of 70% ethanol to the clarified lysate and mix vigorously by vortexing.
5. Apply the total volume (usually 700 µl) from step 4 to the **RNAprep spin column** by decanting or pipetting.
6. Centrifuge at full speed for 90 seconds. Discard the flow-through.
7. Wash the **RNAprep spin column** by adding 250 µL **WB1** and centrifuging at 10000 g for 90 seconds. Discard the flow-through.
8. [Optional] Place the **RNAprep spin column** in a **collection tube** and add 60 µL of RNase-free DNase I solution (0.5U/µl) (not provided) to the centre of the column matrix. Let stand for 15 minute at room temperature.
9. Add 250 µl of **WB1** and centrifuge at full speed for 60 seconds. Discard the flow-through.
10. Add 700 µl of **WB2** and centrifuge at full speed for 1 minute. Discard the flow-through.
11. Again Centrifuge at full speed for 3 minutes. This step helps to dry the **RNAprep spin column**.
12. Place the **RNAprep spin column** into a new, labelled 1.5 microcentrifuge tube and pipet 50-60µl of **RNase-free Water** directly into the. Close the cap and incubate for 1 minute at room temperature.
13. Centrifuge at full speed for 1 minute to elute RNA.
14. Keep eluted RNA on ice at all times and store at -70°C.



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