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_2O. 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	(Reactions)	
Item	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_2O	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)

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 votex 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.