# Blood RNA Isolation Kit

Cat. No.: S-1021-5; 5 applications
Cat. No.: S-1021; 25 applications
Cat. No.: S-1021-1; 50 applications

DENA ZIST ASIA
MOLECULAR BIOLOGY PRODUCTS
www.denazist.ir

## Manual

**Description:** DENAzist blood RNA Isolation Kit contains spin columns and solutions for quick and easy isolation of total RNA from whole blood (citrate, heparin or EDTA added). The unique composition of buffers selectively isolates RNA and leaves the final elute with the least amount of DNA and protein. The isolated total RNA will have the highest quality for any downstream experiment.

VERSION: Sept 2023

### Important instructions before first use

For the 5 application kit, add 2.4 ml ethanol (96%-100%) to the container labeled **BR2**. For the 25 application kit, add 12 ml ethanol (96%-100%) to the container labeled **BR2**. For the 50 application kit, add 24 ml ethanol (96%-100%) to the container labeled **BR2**. Label the container to record that ethanol has been added before first use.

### PROTOCOL for isolation of total RNA from whole blood samples

Before the first use, make sure that ethanol has been added to the container labeled BR2.

**Note:** All steps should be performed in RNase-free conditions.

1. Add 6 ml **BR1** Buffer to a sterile 15 ml (Falcon) tube.
2. Add 2 ml of fresh (or properly stored) whole blood and mix by inversion. Do not vortex.

   *Important: To isolate high-quality RNA, use freshly prepared anti-coagulant-added blood or blood samples that are properly stored (kept at -80°C immediately after sampling).*

3. Incubate the tube at room temperature for 15 minutes with periodic inverting.

   *Note: The isolation can be stopped at this step. The blood samples in BR1 will have great quality RNAs when kept at the fridge for up to 6 hours.*

4. Centrifuge the tube for 10 min at 4000 rpm. Cell debris will be accumulated at the bottom of the tube.
5. Transfer the whole supernatant to a new 15 ml tube. Discard the pellet. Add 1.6 ml chloroform. Shake vigorously. Incubate the tube at room temperature for 3 minutes.
6. Centrifuge the tube for 15 min at 4000 rpm. Three phases will form. Carefully transfer the top phase to a new tube. Discard the interphase and the bottom phase.
7. To the tube containing the supernatant, add half the volume (of supernatant) from ethanol. Mix well by inverting.

-------RNA BINDING TO THE COLUMN------

8. Transfer the above mix from previous step in amounts of 800 µl onto a spin column inserted into a collecting tube (provided by the kit). Centrifuge the tube at 10,000 rpm for 1 minute at room temperature. Discard the flowthrough.
9. Repeat step 8 until all of the solution has been passed through the column.

-------WASHING------

10. Add 500 µl **BR2** to the spin column. Centrifuge at 10,000 rpm for 1 minute at room temperature. Discard the flowthrough.
11. Add 250 µl BR2 to the spin column. Centrifuge at 10,000 rpm for 1 minute at room temperature. Discard the flowthrough.
12. Without adding any solutions, centrifuge the tube one more time at 13,000 rpm for 3 minutes at room temperature.

-------ELUTION-------

13. Transfer the column onto a new 1.5 ml microfuge tube. Add 50 µl **BR3** to the spin column. Incubate at room temperature for 2 minutes. Spin the tube at 8000 rpm for 2 minutes at room temperature.
14. Add the eluted solution from the previous step back onto the center of the spin column. Leave the spin column mounted on the microfuge tube for 2 minutes at room temperature. Centrifuge the spin column mounted on microfuge tube at 13,000 rpm for 2 minutes. The eluted sample will contain total RNA.
15. If necessary, determine RNA concentration and quality by spectrophotometry and gel electrophoresis. Keep aliquots of the sample at -80 °C. ◢

### Kit components:

| | 5 app. | 25 app. | 50 app. |
|---|---|---|---|
| **BR1** | 30 ml | 150 ml | 300 ml |
| **BR2** | 1.8 ml | 9 ml | 18 ml |
| **BR3** | 1 ml | 3 ml | 6 ml |
| **Spin columns** | 5 | 25 | 50 |

Store BR1 at 4 °C, and BR2 and BR3 buffers at room temperature.
**Expiry date:** 24 month after shipping

**Notes: Research Use Only**
This product insert declares that this products has been analyzed and passed the quality control tests at the time of manufacture.