

# Blood RNA Isolation Kit



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



## 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.

Components	Applications		Storage	Shipping	Expiry date
	25	50			
BR1 buffer	150 ml	300 ml	RT	Shipped at ambient temperature.	The kit is fully active until the expiry date indicated on the box, and when all components are stored under the recommended conditions.
BR2 buffer	9 ml	18 ml	RT		
BR3 buffer	3 ml	6 ml	RT		
Spin columns	25	50	RT		

## Important instructions before first use

For 25 application kit, add 12 ml ethanol (96%-100%) to the container labeled BR2.  
 For 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 first use, make sure that ethanol has been added to the container labeled BR2. Add 6 ml BR1 Buffer to a sterile 15 ml (Falcon) tube.
- 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 which are properly stored (kept at -80°C immediately after sampling).
- Incubate the tube at room temperature for 15 minutes with periodic inverting.

- The isolation can be stopped at this step. The blood samples in BR1 will have great quality RNAs when kept at fridge for up to 6 hours.
- Centrifuge the tube for 10 min at 4000 rpm. Cell debris will be accumulated at the bottom of the tube.
  - 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.
  - 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.

### -----RNA BINDING TO COLUMN-----

- To the tube containing the supernatant, add half volume (of supernatant) from ethanol. Mix well by inverting.
- Transfer the solution 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 minutes at room temperature. Discard the flowthrough.
- Repeat step 8 until all of the solution has passed through the column.

### -----WASHING-----

- Add 500 µl BR2 to the spin column. Centrifuge at 8,000 rpm for 1 minute at room temperature. Discard the flowthrough.
- Add 250 µl BR2 to the spin column. Centrifuge at 10,000 rpm for 1 minute at room temperature. Discard the flowthrough.
- Without adding any solutions, centrifuge the tube one more time at 13,000 rpm for 3 minutes at room temperature.

### -----ELUTION-----

- 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.
- Add the eluted solution from the previous step back onto the center of 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.
- If necessary, determine RNA concentration and quality by spectrophotometry and gel electrophoresis. Keep aliquotes of the sample in -80°C. ▲

Please see [denazist.com](http://denazist.com) for troubleshooting and more technical information.

#### 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.