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Fast Blood RNA kit

Product Number: RNK2502

Shipping and Storage

1. Transportation and storage are carried out at room temperature (15°C-25°C). Buffer TRI can be transported at room temperature and stored in the dark at 4°C for a long time after receipt. Even if stored at room temperature for 3 months, it will not affect the quality of use.
2. Avoid prolonged exposure of reagents to air, which can cause volatilization, oxidation, and changes in pH value. After using each solution, the lid should be tightly closed in a timely manner.

Components

Component	Storage	RNK2502 50Preps
10×Buffer TRIB	RT	25mL
Buffer TRI	4°C light avoidance	50mL
Buffer RE	RT	16mL
Buffer RW	RT	10mL
RNase-free H_2O	RT	5mL
RNase free adsorption column RA	RT	50
Collection tube (2mL)	RT	50

Description

First, use red blood cell lysis buffer to lyse fresh anticoagulated whole blood red blood cells to obtain white blood cells. Improved guanidine isothiocyanate/phenol one-step method (TRIzol method) is used to lyse cells and inactivate RNAses. Total RNA is then selectively adsorbed onto the silica matrix membrane in a highly ionized salt state, followed by a series of rapid rinsing centrifugation steps to remove cell metabolites, proteins, and other impurities from the protein solution and rinse solution. Finally, low salt RNase free water is used to elute pure RNA from the silica matrix membrane.

Features

1. The silicon matrix membrane inside the centrifugal adsorption column is entirely made of specially designed adsorption membranes, with minimal differences in adsorption capacity between columns and good repeatability.
2. Combining the advantages of good stability, high purity, and convenient and fast centrifugation column of guanidine isothiocyanate/phenol one-step reagent, RNA can be directly eluted from the centrifugation column without the need for isopropanol precipitation and ethanol washing process, avoiding the problem of excessive drying and difficult dissolution.
3. Buffer TRI corresponds to Thermo Fisher/Invitrogen's TRIzol, which can effectively eliminate genomic contamination. The effect is consistent with the import.
4. Repeated rinsing to remove protein results in higher purity of RNA extraction.
5. Effectively removed the content of 5S in total RNA and improved purity.

Application

Suitable for rapid extraction of high-purity total RNA from whole blood

Note

1. 1. This reagent kit has excellent inhibition effect on RNAse, and all centrifugation steps can be performed at room temperature unless otherwise specified.

2. Buffer TRI and deproteinized PE contain irritating and harmful compounds. Latex gloves should be worn during operation to avoid contact with skin, eyes, and clothing. If it comes into contact with the skin or eyes, rinse with plenty of water or saline solution.
3. 3. Considering environmental issues, this reagent kit does not contain the commonly used laboratory reagent chloroform, and chloroform needs to be prepared before use.
4. 4. Conventional agarose gel electrophoresis and formamide denaturing gel electrophoresis can be used to analyze the quality of RNA. A good RNA product should show two distinct dominant ribosomal RNA bands after denaturing electrophoresis, namely~5 Kb (28S) and~2 Kb (18S) (note that in regular agarose electrophoresis, 28S and 18S are approximately located at 2 Kb and 1 Kb positions, and the position of the electrophoretic band may not be fixed depending on the secondary structure of the RNA space), with a band brightness ratio of about 2:1. Sometimes,~0.1 Kb and 0.3 Kb (5S, tRNA) bands can also be seen. But sometimes it is normal to see 4 or 5 bands depending on different species, such as certain plant tissues. If immature RNA precursors or heterogeneous nuclear RNA or small nuclear RNA are extracted, discontinuous high molecular weight bands between 7 Kb and 15 Kb may also be seen
5. When detecting the OD260/OD280 absorbance ratio, if RNA samples need to be diluted, TE (pH 8) should be used. If diluted with water for detection, due to the generally low water ion strength and pH value, OD280 will increase, resulting in a decrease in the ratio.
6. After adding Buffer TRI homogenate, the sample can be stored at -60°C to -70°C for more than one month before adding chloroform.

Protocol

Tip: 1) Before the first use, please add the specified amount of ethanol to the Buffer RW bottle and 70% ethanol bottle!
2)Dilute 10×Buffer TRIB with DEPC treated water to 1×before use.

1. Add 1 volume (0.5-1.0mL) of fresh blood with various anticoagulants (mixed upside down) and 3 volumes of Buffer TRIB to an RNase free centrifuge tube of appropriate size. Mix upside down and gently tap the tube wall to ensure uniformity.
   The number of white blood cells in the patient's blood sample may significantly increase or decrease, and the processing volume should be appropriately increased or reduced.
2. Let it stand at room temperature for 10 minutes (during which it should be reversed and gently mixed several times to help lyse red blood cells).
   If RNA degradation is severe, it can be broken down on ice, but the time can be longer to fully break down.
3. Centrifuge at 12000 rpm for 20 seconds, discard the red supernatant, and carefully aspirate as much supernatant as possible (be careful not to aspirate cell clusters at the bottom of the tube), leaving intact white blood cell clusters at the bottom of the tube.
   After centrifugation, white white blood cell clusters should be seen at the bottom of the tube, and there may also be some red blood cell fragments and white blood cell clusters together. However, if most of the red blood cell clusters are seen, it indicates that red blood cell lysis is not sufficient. Buffer TRIB should be added to resuspend the cell clusters and repeat steps 2 and 3.
   Try to absorb and discard the supernatant as much as possible. Excessive residue will dilute the Buffer TRIB, causing abnormal cracking and binding, resulting in a decrease in yield and purity
4. Vortex or light bullet tube walls will completely loosen and resuspend white blood cell precipitates. Add 1mL of Buffer TRI and repeatedly blow with a pipette to lyse the cells.
5. Shake the homogenate sample vigorously and mix well. Incubate at 15-30℃ for 5 minutes to completely decompose the ribosomes.
6. Add 0.2mL of chloroform to every 1mL of Buffer TRI. Close the sample tube tightly, shake vigorously for 15 seconds, and let it sit at room temperature for 2 minutes.
7. Centrifuge at 4 ℃ and 12000 rpm for 10 minutes, and the sample will be divided into three layers: the lower organic phase, the middle layer, and the upper colorless aqueous phase. RNA is present in the aqueous phase. The capacity of the aqueous layer is approximately 60% of the volume of Buffer TRI added. Transfer the aqueous phase to a new tube for the next step of operation.

8. 8. Add half the volume of the aqueous phase, which is 0.5 times the volume of anhydrous ethanol, and mix well (precipitation may occur at this time). The obtained solution and possible precipitate are transferred into the adsorption column RA together (the adsorption column is enclosed in a collection tube. If it is not possible to add all the solution and mixture into the adsorption column RA at once, please transfer them into the adsorption column RA in two separate times.) Centrifuge at 12000 rpm for 30 seconds at room temperature (all steps below are at room temperature), discard the waste liquid, and replace the adsorption column with a recovery header.
9. Add 500μL Buffer RE(please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, discard the waste liquid.
10. Add 500μL Buffer RW (please check if anhydrous ethanol has been added first!), centrifuge at 12000 rpm for 15 seconds, and discard the waste liquid.
11. Repeat step 10 once.
12. Put the adsorption column RA back into the empty collection tube, centrifuge at 12000rpm for 2 minutes, and try to remove Buffer RW as much as possible to avoid residual ethanol in Buffer RW inhibiting downstream reactions.
13. Remove the adsorption column RA and place it in an RNase free centrifuge tube. Add 30-50μL of RNase free H_2O to the middle of the adsorption membrane according to the expected RNA yield. Let it stand at room temperature for 2 minutes and centrifuge at 12000rpm for 1 minute.

    The recommended elution volume is not less than 30μL, as a small volume can affect the efficiency of nucleic acid recovery.

    The following steps can help increase the concentration of RNA products: RNase free ddH_2O is preheated at 70-90°C before elution; Add the first eluent back to the adsorption column for a second elution.