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Fast Blood RNA kit

Product Number: RNK2501

Shipping and Storage

1. All solutions should be clear. If the ambient temperature is low, the solution may form precipitates and should not be used directly. It can be heated in a 37℃ water bath for a few minutes to restore clarity.
2. Inappropriate storage at low temperatures (4℃ or -20℃) can cause solution precipitation, affecting the effectiveness of use. Therefore, transportation and storage are carried out at room temperature (15℃ -25℃). Buffer RL can be transported at room temperature and stored in dark at 4 ℃ upon receipt.
3. To avoid volatilization, oxidation, and pH changes caused by prolonged exposure of reagents to the air, each solution should be covered tightly in a timely manner after use.

Components

Component	Storage	RNK2501 20Preps	RNK2502 50Preps
10×Buffer RLB	RT	10 ml	20ml
Buffer RL	4°C light avoidance	25 ml	50 ml
Buffer RE	RT	15ml	25 ml
Buffer RW	RT	5ml	10 ml
RNase-free H_2O	RT	10 ml	10 ml
70% ethanol	RT	4ml RNase-free H_2O	9ml RNase-free H_2O
RNase free adsorption column RA	RT	20	50
Collection tube (2ml)	RT	20	50

Description

The improved guanidine isothiocyanate/phenol one-step method cleaves cells and inactivates RNA enzymes. Then, the total RNA selectively adsorbs onto the silica matrix membrane in a highly dissociated salt state. Through a series of rapid rinsing centrifugation steps, Buffer RE and Buffer RW remove cell metabolites, proteins, and other impurities. Finally, low salt RNase free H2O elutes 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 from world-renowned imported companies, with minimal differences in adsorption capacity between columns and good repeatability. Overcoming the drawback of unstable membrane quality in domestic reagent kits.
2. Combining the advantages of good stability, high purity, and convenient and fast centrifugation column of guanidine isothiocyanate/phenol one-step method reagents, RNA can be directly eluted from the centrifugation column without the need for isopropanol precipitation and ethanol washing process, avoiding the problem of over drying and difficult dissolution.
3. The unique Buffer RL formula can effectively eliminate genomic contamination.
4. Multiple rinses to remove proteins result in higher purity of extracted RNA.
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

For Research Use Only

1. Before the first use, please add the specified amount of ethanol to the Buffer RW bottle and 70% ethanol bottle. After adding, please mark the added ethanol with a check mark in a timely manner to avoid adding it multiple times!
2. All centrifugation steps, unless otherwise specified, are carried out at room temperature. Use a traditional desktop centrifuge that can reach a speed of 13000rpm, such as Eppendorf5415C or a similar centrifuge.
3. Buffer RL and Buffer RE contain irritating and harmful compounds. Latex gloves should be used during operation to avoid contact with skin, eyes, and clothing. If it gets on the skin or eyes, rinse with plenty of water or physiological saline.
4. Considering environmental protection issues, this reagent kit does not contain commonly used laboratory reagent chloroform. Users need to bring their own chloroform before use.
5. Conventional agarose gel electrophoresis and 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 electrophoresis, namely~5Kb (28S) and~2Kb (18S), with a band brightness ratio of approximately 2:1. Sometimes~0.1kb and 0.3Kb (5S, tRNA) bands can also be seen. But sometimes it is normal to see 4-5 bands depending on different species, such as certain plant tissues. If RNA precursors are not mature or uneven nuclear RNA or small nuclear RNA is extracted, discontinuous high molecular weight bands between 7Kb and 15Kb may also be seen.
6. A low value will cause an increase in OD280, resulting in a decrease in the ratio.
7. After adding buffer RL homogenate and before adding chloroform, the sample can be stored at -60 ℃ -70 ℃ for more than one month.
8. Regarding trace residues of DNA:

   Generally speaking, any total RNA extraction reagent cannot completely avoid the trace residue of DNA during the extraction process. In most RT-PCR amplification processes, the extremely small amount of DNA residue (usually not visible under electrophoresis EB staining and UV light observation) does not have a significant impact. If strict mRNA expression analysis, such as fluorescence quantitative PCR, is required, we recommend that when selecting templates and primers:

   8.1. Select primers that cross introns to pass through the connecting regions in mRNA, so that DNA cannot serve as a template for amplification reactions.

   8.2. Select primer pairs with different sizes of amplified products on genomic DNA and cDNA.

   8.3. Treat the RNA extract with DNase I of RNase free.

   8.4. Before rinsing the buffer RE, DNase I digestion treatment is directly performed on the adsorption column RA.

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 RLB 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 RLB 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 RLB 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 RLB, 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 RL 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 RL. 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 RL added. Transfer the aqueous phase to a new tube for the next step of operation.
8. Add an equal volume of 70% ethanol (please check if anhydrous ethanol has been added!), invert and mix well (precipitation may occur at this time). The obtained solution and possible precipitate are transferred together into the adsorption column RA (the adsorption column is nested inside the collection tube).
9. Centrifuge at 12000rpm for 45 seconds, discard the waste liquid, and reinstall the adsorption column into the recovery manifold.
10. Add 500μL Buffer RE, centrifuge at 12000rpm for 45 seconds, discard the waste liquid.
11. Join 500μL Buffer RW (please check if anhydrous ethanol has been added first!), centrifuge at 12000 rpm for 45 seconds, and discard the waste liquid.
12. Join 500μL Buffer RW, centrifuge at 12000rpm for 45 seconds, discard the waste liquid.
13. Put the adsorption column RA back into the empty collection tube, centrifuge at 13000rpm for 2 minutes, and try to remove Buffer RW as much as possible to avoid residual ethanol in Buffer RW inhibiting downstream reactions.
14. Take out the adsorption column RA and place it in an RNase free centrifuge tube. Add 50-80μL of RNase free H_2O to the middle of the adsorption membrane according to the expected RNA production (heating in a 65-70℃ water bath beforehand is more effective). Leave at room temperature for 2 minutes and centrifuge at 12000rpm for 1 minute. If more RNA is needed, the obtained solution can be re added to the centrifuge adsorption column and centrifuged for 1 minute, or an additional 30μL of RNase free H_2O can be added and centrifuged for 1 minute, and the two elutions can be combined.

    The larger the elution volume, the higher the elution efficiency. If a higher RNA concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 30μL. Small volume reduces RNA elution efficiency and RNA production