MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

EASYspin Fast Blood RNA Kit

Product Number: RNK0602

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 ℃).
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	RNK0602 50 Preps
10×Buffer RLB	RT	25 ml
Buffer RLT	RT	30 ml
Buffer RW1	RT	40 ml
Buffer RW	RT	10 ml
70% ethanol	RT	9ml RNase-free H_2O
RNase-free H2O	RT	10 ml
RNase free adsorption column RA and collection tube	RT	50

Description

Buffer RLB selectively cleaves red blood cells, followed by unique Buffer RLT/ β- Mercaptoethanol rapidly cleaves white blood cells and inactivates cell RNA enzymes. After adjusting the binding conditions with ethanol, RNA selectively adsorbs onto the silica matrix membrane in a highly dissociated salt state. Through a series of rapid rinsing centrifugation steps, Buffer RW1 and Buffer RW remove impurities such as cell metabolites and proteins. Finally, low salt RNase free H2O elutes pure RNA from the silica matrix membrane.

Features

1. No toxic reagents such as phenol/chloroform are required, and no steps such as ethanol precipitation are required.
2. The repeatedly optimized Buffer RLB formula achieves fast and complete cracking effect.
3. Fast and simple, the operation of a single sample can generally be completed within 40 minutes.
4. Multiple column washes ensure high purity, with a typical OD260/OD280 ratio of 1.9-2.1 and almost no DNA residue. It can be used for RT-PCR, Northern blot, and various experiments.

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 13000 rpm.
2. Self prepared ethanol is required, β- Mercaptoethanol.
3. Buffer RLT and Buffer RW1 contain irritating compounds, and latex gloves should be worn 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. Regarding trace residues of DNA:
5. Generally speaking, any total RNA extraction reagent cannot completely avoid trace DNA residues during the extraction

process. Our company's EASYspin series RNA extraction products, due to the use of our unique buffer system and the selection of adsorption membranes with special adsorption capabilities, do not have a significant impact on extremely small amounts of DNA residues in most RT-PCR amplification processes. If strict mRNA expression level analysis, such as fluorescence quantitative PCR, is required, We suggest that when selecting templates and primers:

6. Select primers that cross introns to pass through the connecting regions in mRNA, so that DNA cannot serve as a template for amplification reactions. Alternatively, primer pairs with different sizes of amplified products on genomic DNA and cDNA can be selected. Shorten the extension time to prevent the DNA source template from participating in the amplification reaction.
7. Treat the RNA extract with DNase I of RNase free. This reagent kit can also be used for RNA cleaning after DNase I treatment. Please contact us for specific operating instructions.
8. Before rinsing buffer RW1, perform DNase I treatment (RNK3401: DNase Digestion Kit) directly on the adsorption column RA. Refer to Appendix 1.

Protocol

Tips:1)Please add the specified amount of anhydrous ethanol to the Buffer RW bottle and 70% ethanol bottle before first use!

2)Dilute 10×Buffer RLB with DEPC treated water to 1×before use.

3)Add buffer RLT before operation β- Mercaptoethanol to final concentration of 1%, add 10ul to 1 ml RLT β-Mercaptoethanol. It is best to use and configure this Buffer RLT as it is. The prepared buffer RLT can be stored at 4 ℃ for one month.

1. Add 1 volume (<1.5 ml) 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 bounce the tube wall to ensure uniformity.
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, and a decrease in yield and purity.
4. Vortex or light bulb tube wall will completely loosen and resuspend white blood cell precipitation. Add 350ul (<0.5ml whole blood) or 600ul (0.5-1.5ml whole blood) Buffer RLT, blow and mix well, then vigorously shake by hand for 20 seconds to fully lyse.

   The normal white blood cell count of the patient is 4000-7000/ul. If the white blood cell count in the blood sample may increase significantly, the processing volume should be appropriately reduced. Alternatively, buffer RTL can be added in a ratio of 350ul (<$2x10^6$ white blood cells) or 600ul ($2x10^6$-$1x10^7$ white blood cells).
5. Blow and mix with a suction head to aid in cracking or vigorous vortex shaking until satisfactory homogenization results are obtained (or electric homogenization for 30 seconds), which can shear DNA, reduce viscosity, and increase yield.
6. Accurately estimate the volume of the lysate by adding an equal volume of 70% ethanol (please check if anhydrous ethanol has been added first!). At this time, precipitation may occur, but it does not affect the extraction process. Immediately blow and mix well, do not centrifuge.
7. Immediately add the mixture (less than 700ul each time, which can be added in two separate batches) to an adsorption column RA, centrifuge at 13000 rpm for 60 seconds, and discard the waste liquid.
8. Add 700ul Buffer RW1, let it stand at room temperature for 1 minute, centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
9. Add 500ul of Buffer RW (please check if anhydrous ethanol has been added first!), centrifuge at 12000 rpm for 30 seconds, and

discard the waste liquid. Add 500ul Buffer RW and repeat.

10. Put the adsorption column RA back into the empty collection tube, centrifuge at 13000 rpm for 2 minutes, and try to remove Buffer RW as much as possible to avoid residual ethanol in Buffer RW inhibiting downstream reactions.
11. Take out the adsorption column RA and place it in an RNase free centrifuge tube. Add 30-50ul of RNase free water to the middle of the adsorption membrane according to the expected RNA production (heating in a 70-80 ℃ water bath beforehand is more effective), leave at room temperature for 1 minute, and centrifuge at 12000 rpm for 1 minute.
12. If extracting whole blood>0.5 ml or>$2x10^6$ white blood cells, add 30-50 ul of RNase free water and repeat step 11 to obtain more RNA, or use the first eluent to add back to the adsorption column and repeat step again (if high RNA concentration is required).

The concentration of RNA elution solution after two washes is high. If the combined elution solution produces 15-30% higher RNA production than the former after two washes, but the concentration is lower. Users can choose according to their needs.

Appendix 1: DNA Enzyme Column Digestion

(please refer to RNK3401: DNase Digestion Kit manual for details)

1. Follow the EASYspin Fast Blood RNA Kit steps listed earlier until protocol step 7 is completed.
2. Take 45ul of DNase I buffer and 5ul of RNase free DNase I and gently blow them in a centrifuge tube to mix well to form a working solution (when processing multiple centrifuge columns, prepare the working solution proportionally).
3. Add 350ul Buffer RW1 to the adsorption column RA, centrifuge at 12000 rpm for 30 seconds, discard the waste liquid, and place the adsorption column in the recovery manifold.
4. Add 50ul of DNase I working solution to the center of the adsorption column RA and let it stand at room temperature (20 ℃ -30 ℃) for 15 minutes. Pay attention to directly immerse the working liquid droplets in the center of the membrane and make full contact with the membrane around the membrane. Do not let the working liquid droplets hang on the O-shaped gasket or centrifugal column wall, or hang on the gasket without sufficient contact with the membrane.
5. Add 350ul Buffer RW1 to the adsorption column RA, centrifuge at 12000 rpm for 30-60 seconds, discard the waste liquid, and place the adsorption column in the recovery manifold.
6. Follow protocol step 9 to complete the subsequent steps.