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EASYspin Fibrous Tissue Fast RNA Kit

Product Number: RNK4401

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 reducing activity and facilitate transportation, protease K is provided as a freeze-dried powder. After receiving it, it can be briefly centrifuged and dissolved in 0.25ml (20 times) and 0.5ml (50 times) of RNase free H_2O. Because repeated freeze-thaw cycles may reduce enzyme activity, after dissolution, it can be immediately packaged and frozen according to the amount used each time, and stored at -20℃.
4. 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.

Component

Component	Storage	RNK4401 20preps	RNK4402 50preps
Buffer RLT	RT	20ml	50 ml
Buffer RW1	RT	15 ml	40 ml
Buffer RW	RT	5 ml	10ml
RNase-free H_2O	RT	20 ml	40 ml
Protease K	4℃	10mg	20mg
RNase free adsorption column RA and collection tube	RT	20	50

Description

Unique Buffer RLT/ β-Mercaptoethanol rapidly cleaves 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 cellular metabolites and proteins. Finally, low salt RNase free H2O washes pure RNA off the silica matrix membrane.

Feature

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. No toxic reagents such as phenol and chloroform are needed, and no steps such as ethanol precipitation are required.
3. Fast and simple, the operation of a single sample can generally be completed within 30 minutes.
4. Multiple column washes ensure high purity, with a typical OD260/OD280 ratio of 1.9-2.0 and almost no DNA residue. It can be used for RT-PCR, Northern blot, and various experiments.

Applications

Suitable for rapid extraction of tissue RNA rich in heart, skeletal muscle, blood vessels, trachea, skin, and other fibers

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge that can reach a speed of

13000 rpm, such as Eppendorf 5415C or a similar centrifuge.

2. Self prepared ethanol is required, β-Mercaptoethanol, disposable syringe, mortar, water bath, etc.
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. To prevent RNase pollution, attention should be paid to the following aspects:
   - 4.1. Regularly replace with new gloves. Because the skin often carries bacteria, it may lead to RNase contamination.
   - 4.2. Use RNase free plastic products and gun heads to avoid cross contamination.
   - 4.3. RNA is not degraded by RNase in Buffer RLT. However, plastic and glassware without RNase should be used during the extraction and subsequent processing. Glassware can be baked at 150 ℃ for 4 hours, while plastic containers can be soaked in 0.5M NaOH for 10 minutes, then thoroughly washed with water and sterilized to remove RNase.
   - 4.4. Prepare the solution using water without RNase. (Add water to a clean glass bottle, add DEPC to the final concentration of 0.1% (v/v), leave at 37 ℃ overnight, and sterilize under high pressure.)
5. Regarding trace residues of DNA:

   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, have minimal impact on the extremely small amount of DNA residues in most RT-PCR amplification processes (usually invisible under electrophoretic EB staining and UV light observation). If strict mRNA expression level analysis, such as fluorescence quantitative PCR, is required, we recommend that when selecting templates and primers:
   - 5.1. Select primers that cross introns to pass through the connecting regions in mRNA, so that DNA cannot serve as a template for amplification reactions.
   - 5.2. Select primer pairs with different sizes of amplified products on genomic DNA and cDNA.
   - 5.3. 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.
   - 5.4. Before rinsing buffer RW1, perform DNase I treatment directly on the adsorption column RA. Please contact us to request specific operating instructions.
6. RNA purity and concentration detection:
   - 6.1. Integrity: RNA can be detected by ordinary agarose gel electrophoresis (electrophoresis conditions: gel concentration 1.2%; 0.5×TBE electrophoresis buffer; 150v, 15min). Due to 70% -80% of RNA in cells being rRNA, very clear rRNA bands should be visible under UV after electrophoresis. Animal rRNAs are approximately 5 kb and 2 kb in size, equivalent to 28 S and 18 S rRNAs, respectively. The maximum rRNA brightness in animal RNA samples should be 1.5-2.0 times the brightness of the second largest rRNA, otherwise it indicates degradation of the RNA sample. The appearance of dispersed flakes or disappearance of bands indicates severe degradation of the sample.
   - 6.2. Purity: The OD260/OD280 ratio is an indicator of the degree of protein contamination. High quality RNA, OD260/OD280 reading (10mMTris, pH 7.5) between 1.8-2.1. The OD260/OD280 reading is affected by the pH value of the solution used for measurement. Assuming that the OD260/OD280 readings for the same RNA sample are between 1.8-2.1 in a 10mM Tris, pH 7.5 solution, and between 1.5-1.9 in an aqueous solution, this does not mean that the RNA is impure.
   - 6.3. Concentration: Take a certain amount of RNA extract, dilute it n times with RNase free water, adjust the spectrophotometer to zero with RNase free water, take the diluent for OD260 and OD280 measurements, and calculate the RNA concentration according to the following formula: final concentration (ng/μl)=(OD260)×(dilution n)×40.

Protocol(Please read the precautions before the experiment)

Tip: 1) Before the first use, please add the specified amount of anhydrous ethanol to the Buffer RW bottle and 70% ethanol bottle!

2) Add buffer RLT before operation β- Mercaptoethanol to final concentration of 1%, add 10μl 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. **Fully crushing the homogenate (very important, otherwise it will significantly reduce production)**

1.1. **Electric homogenization (strongly recommended, with the highest yield and most stable results):** Take about 10-20mg (<30mg) of fresh tissue, add 300μl Buffer RLT, and thoroughly crush the homogenized tissue cells using an electric blade homogenizer (Rotor Stator such as TissueRupter) or an electric glass bead grinder (Bead Mill such as TissueLyser) according to the instructions for machine use.

1.2. **Liquid nitrogen grinding+homogenization:** After grinding the tissue into fine powder in liquid nitrogen, take an appropriate amount of 10-20mg (<30mg) of tissue fine powder and transfer it into a 1.5ml centrifuge tube containing 300μl Buffer RLT. Shake vigorously by hand for 20 seconds to fully crack. Using a disposable 1 ml (with a 0.9mm needle) syringe with a blunt needle, the lysate can be aspirated 10 times or until satisfactory homogenization results are obtained (or electric homogenization for 30 seconds), which can cut DNA, reduce viscosity, and increase yield. Transfer the homogenate into a new centrifuge tube.

1.3. **Grind homogenate in a mortar:** Take an appropriate amount of 10-20mg (<30mg) of tissue powder and put it into a small mortar. Quickly add 300μl Buffer RLT and grind the homogenate thoroughly at room temperature. Transfer the homogenate into a new centrifuge tube.

Note: If the loss of homogenate is significant when it is immersed in the mortar, the initial tissue dosage and Buffer RLT dosage can be appropriately increased in proportion. In addition, it is also possible to first grind the tissue into fine powder with liquid nitrogen, and then add Buffer RLT to thoroughly grind the slurry when the liquid nitrogen has just evaporated, which can improve the grinding effect.

2. Suck 590μl of RNase free H2O into the homogenate. Add 10μl of protease K, pipette and beat well.
3. Take a water bath at 55 ℃ for 10 minutes.
4. Centrifuge at 13000rpm for 5 minutes at room temperature. This will form a small amount of tissue debris precipitation, and a small amount of floating debris may be seen at the top of the supernatant.
5. Transfer the supernatant to a new 1.5ml centrifuge tube.

Note: Do not introduce sediment during transfer, and the pipette tip must be placed under the upper clear floating object. Floating debris may usually adhere to the outer wall of the gun head, so be careful not to introduce it into the centrifuge tube.

6. Accurately estimate the volume of the lysate (supernatant) by adding 0.5 volume of anhydrous ethanol (usually 450μl). Precipitation may occur at this time, but it does not affect the extraction process. Immediately blow and mix well, without centrifugation.
7. Immediately add the mixture (less than 700μl each time, can be added in two batches) to the same adsorption column RA, centrifuge at 13000 rpm for 60 seconds (the adsorption column is placed in the collection tube), and discard the waste liquid.
8. Add 700μl Buffer RW1, let it stand at room temperature for 30 seconds, centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.

If there is significant DNA residue, it can be left at room temperature for 5 minutes after adding Buffer RW1 and then centrifuged.

9. Add 500μl 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 500μl 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 30-50μl of RNase free water to the middle of the adsorption membrane according to the expected RNA yield (heating in a 70-90 ℃ water bath beforehand can increase yield). Leave at room temperature for 1 minute and centrifuge at 12000 rpm for 1 minute.
12. Optional: Add the first elution solution back to the adsorption column and repeat the steps once (if high RNA concentration is required).

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The RNA concentration in the RNA elution solution after two washes can be appropriately increased. If the expected RNA production is greater than 30ug, 30-50μl of RNase free water can be added and the steps can be repeated to merge two eluents, which can increase the production by 15-30%, but the concentration will be slightly reduced.