MEBEP BIOSCIENCE
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EZ spin Plus Tissue/cell Fast RNA kit

Product Number: RNK2802

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	RNK2802 50 Preps
Buffer RLT Plus	RT	50ml
Buffer RW1	RT	40ml
Buffer RW	RT	10ml
RNase-free H_2O	RT	10ml
70% ethanol	RT	9ml RNase-free H_2O
Genomic DNA clearance column and collection tube	RT	50
RNase free adsorption column RA and collection tube	RT	50

Description

On the basis of the exclusive launch of EZ spin phenol free and chloroform based rapid RNA extraction technology, our company has also independently developed a genome DNA clearance column technology to ensure effective removal of gDNA residues. The obtained RNA does not require DNase digestion and can be directly used for reverse transcription PCR, fluorescence quantitative PCR and other experiments. The unique Buffer RLT Plus rapidly cleaves cells and inactivates cellular RNA enzymes, and then the mixture is cleaved through a genomic DNA clearance column, where genomic DNA is cleared and RNA penetrates through filtration. After adjusting the binding conditions with ethanol, the filtered RNA selectively adsorbs onto the silica matrix membrane in a highly dissociated salt state. Then, 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 H_2O elutes pure RNA from the silica matrix membrane.

Features

1. Completely do not use toxic reagents such as phenol, chloroform, Beta mercaptoethanol, and do not require steps such as ethanol precipitation.
2. Fast and simple, the operation of a single cell sample can generally be completed within 15 minutes.
3. The exclusive development of genomic DNA clearance column technology ensures effective clearance of gDNA residues. The obtained RNA does not require DNase digestion and can be directly used for reverse transcription PCR, fluorescence quantitative PCR and other experiments.
4. Multiple column washes ensure high purity, with a typical OD260/OD280 ratio of 2.1-2.2 (the ratio of 100% pure RNA is generally around 2.2. Many companies' products have reduced ratios due to residual proteins or DNA, which cannot meet the purity standard of 2.2. Therefore, reducing the requirement by 1.9-2.0 is sufficient for use, but our product standards can generally reach high levels of 2.1-2.2 purity).

Application

Suitable for rapid extraction of total RNA from ordinary animal cells and easily lysed animal tissues, using unique genomic DNA clearance column technology to ensure effective clearance of gDNA residues, without the need for DNase digestion, RNA can be directly used for reverse transcription fluorescence quantitative PCR, Downstream experiments such as Northern blot.

Note

1. **All centrifugation steps are completed at room temperature,** using a desktop centrifuge with a speed of up to 13000 rpm is sufficient.
2. **The sample processing capacity should never exceed the processing capacity of the genome adsorption column DA and RNA adsorption column RA, otherwise it may cause DNA residue or yield reduction.** There is a significant difference in RNA/DNA among different types of tissue cells, for example, the thymus and spleen have abundant DNA content, exceeding 5mg will exceed the column processing capacity. COS cells have abundant RNA content, exceeding 3×10^6 cells will exceed the column adsorption capacity. **So when starting to explore the experimental conditions, if the DNA/RNA content of the sample is not clear, it is better to use a smaller sample processing volume, such as cells not exceeding 3-4×10^6 and tissues not exceeding 10mg. In the future, the processing capacity will be increased or decreased based on the sample testing situation.**
3. Buffer RLT Plus and deproteinized solution RW1 contain guanidine hydrochloride/guanidine isothiocyanate compounds. **When operating, latex gloves should be worn 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 will not be degraded by RNase in Buffer RLT Plus. 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 residues of DNA during the extraction process (DNase digestion cannot achieve 100% residue free). Our company's EZ spin Plus Tissue/cell Fast RNA kit, due to its unique buffer system and genomic DNA clearance column technology, has cleared the vast majority of DNA and does not require DNase digestion. It can be directly used for reverse transcription PCR and fluorescence quantitative PCR. In some special cases, such as residual DNA caused by excessive DNA content or strict mRNA expression analysis requiring fluorescence quantitative PCR, we suggest that when selecting templates and primers:

   5.1. Select primers that cross introns to cross the connections 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 RNA extract with DNase I of RNase free to improve efficacy. 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.
6. RNA purity and concentration detection
   6.1. Integrity:RNA can be detected for integrity by ordinary agarose gel electrophoresis (electrophoresis conditions: gel concentration 1.2%; 0.5TBE 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 2 kb and 1 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

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appearance of small dispersed flakes or disappearance of bands indicates severe degradation of the sample. However, it should be noted that the differentiation is due to the degradation of the extracted RNA sample itself,The extracted RNA is still intact, but degraded during the electrophoresis process.

6.2. Purity: The OD260/OD280 ratio is a reference indicator for measuring the degree of protein contamination. High quality RNA, OD260/OD280 reading between 2.1 and 2.2. The ratio of 100% pure RNA is generally around 2.2 (the ratio of 100% pure RNA is usually around 2.2). Many companies produce products due to the presence of residual proteins or DNA,Causing a decrease in the ratio, the purity standard of 2.2 cannot be achieved, so reducing the requirement by 1.9-2.0 is sufficient for use. However, our product standards can generally reach high levels of purity standards of 2.1-2.2. The OD260/OD280 reading is influenced by the machine used for measurement and also by the pH value of the dilution solution used for measurement. Microspectrophotometers generally do not require dilution and are not affected by the pH value of the diluted solution. However, for the same RNA sample, if the machine requires dilution during measurement, assuming that the OD260/OD280 reading measured in a 10mM Tris, pH 7.5 dilution solution is between 1.9-2.1, and the reading measured in an aqueous solution may be between 1.5-1.9, this does not mean that the RNA is impure

6.3. Take a certain amount of RNA extract, dilute it n times with RNase free water, set 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)

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

1. Cultivate cells

1.1. **Adhering cells:** do not require digestion, thoroughly aspirate the culture liquid, and directly add the recommended amount of Buffer RLT Plus (see Appendix 1) for repeated blowing and cell lysis; For culture containers that are not convenient for direct lysis, cells can be scraped off with a cell scraper or collected into a 1.5ml centrifuge tube after trypsin digestion.

1.2. **Suspension cells:** Collect<10^7 suspension cells into a 1.5ml centrifuge tube.

1.2.1. Centrifuge at 13000rpm for 10 seconds (or 300g for 5 minutes) to allow the cells to precipitate. Completely aspirate and discard the supernatant, leaving behind cell clusters. Note that incomplete disposal of the supernatant will dilute the lysate, leading to a decrease in yield and purity.

1.2.2. Gently flick the bottom of the centrifuge tube to loosen cell sedimentation, add 350μl Buffer RLT Plus (<5×10^6 cells) or 600μl (5×10^6 -1×10^7cells), repeatedly blow and thoroughly lyse with a pipette until no cell clusters are visible

1.2.3. Add all the lysis mixture to the DNA clearance column (the clearance column is placed in the collection tube).

1.2.4. Immediately proceed to **step 3** of the operation

2. Animal tissues (such as mouse liver and brain)

2.1. **Homogenizer homogenization:** After adding 350μl (<20mg tissue) or 600μl (20-30mg tissue) of Buffer RLT Plus to fresh tissue, the tissue is thoroughly ground and homogenized using a glass or electric homogenizer.

2.2. **Liquid nitrogen grinding+homogenization:** After grinding the tissue into fine powder in liquid nitrogen, take an appropriate amount of tissue fine powder (20mg/30mg) and transfer it into a 1.5ml centrifuge tube containing 350μl/600μl Buffer RLT Plus. Shake vigorously for 20 seconds. For difficult to lyse samples, use a pipette to repeatedly blow the homogenization.

Note: If there are too many insoluble fragments after grinding the homogenate, the cracked product after homogenization can be centrifuged at 13000rpm for 3 minutes to precipitate any fragments or insoluble substances that may be difficult to crack. Add the supernatant to the DNA clearance column (place the clearance column in the collection tube).

2.3. Add the evenly ground homogenate to the DNA clearance column (place the clearance column in the collection tube).

2.4. Immediately proceed to **step 3** of the operation.

3. Immediately centrifuge at 13000 rpm for 1 minute and retain the filtrate (RNA in the filtrate). **Ensure that all the liquid is filtered through after centrifugation and there is no residue on the membrane. If necessary, increase the centrifugation force and time.**

4. More accurate estimation of filtrate volume (usually 350μl/600μl). When filtering, the lost volume should be subtracted, and the filtrate can be pipetted to estimate the volume. Add an equal volume of 70% ethanol (**please check if anhydrous ethanol has been add**ed first!). At this time, precipitation may occur, but it does not affect the extraction process. Immediately blow and mix well, do not centrifuge.

5. Immediately add the mixture (less than 720μl each time, which can be added in two separate batches) to an adsorption column RA, centrifuge at 13000 rpm for 30 seconds, and discard the waste liquid.

6. Add 700μl to Buffer RW1, let it stand at room temperature for 30 seconds, centrifuge at 13000rpm for 30 seconds, and discard the waste liquid

7. Add 500μl of Buffer RW (**please check if anhydrous ethanol has been added first!)**, centrifuge at 13000 rpm for 30 seconds, and discard the waste liquid. Join 500μl Buffer RW, repeat it.

8. 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.

9. Take out the adsorption column RA and place it in a clean 1.5ml centrifuge tube. Add 30-50μl of RNase free water to **the middle of the adsorption membrane** according to the expected RNA production. Leave it at room temperature for 1 minute and centrifuge at 1,3000 rpm for 1 minute to obtain the RNA solution.

The volume of the elution buffer should not be less than 30μl, as a small volume can affect the recovery efficiency. If a higher concentration of RNA is required, add the centrifuged RNA solution back to the adsorption column and repeat the elution process.

Appendix 1: Table of cell quantity in adherent culture

Cultivating vessels	Bottom area (cm^2)	Add the amount of culture medium (ml)	The amount of added culture medium
24 well culture plate	2	1.0	5×10^5
6 well culture plate	9.6	2.5	2.5×10^6
3.5cm glass garden	8	3.0	2.0×10^6
6cm glass garden	21	5.0	5.2×10^6
25cm Plastic culture bottle	25	5.0	5.2×106
100ml Plastic culture bottle	33	10.0	7.6×10^6

Note: In general, add 350μl Buffer RLT Plus to a 3.5cm diameter culture dish or smaller culture container, and 600μl Buffer RLT Plus to a 6cm diameter culture dish or larger culture container. The maximum processing capacity does not exceed 10^7 cells