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EASYspin RNA Mini Kit

Product Number: RNK0701

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	RNK0701 50 Preps
Buffer RLT	RT	50ml
Buffer RW1	RT	40 ml
Buffer RW	RT	10ml
RNase-free H2O	RT	10 ml
70% ethanol	RT	9ml RNase-free H_2O
RNase free adsorption column RA and collection tube	RT	50

Description

The unique Buffer RLT rapidly cleaves cells and inactivates cellular 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 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 10 minutes.
3. 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).

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 RNA adsorption column RA processing capacity, otherwise it may actually lead to a decrease in yield. There is a significant difference in RNA content among different types of tissue cells, and COS cells have abundant RNA content. Cells exceeding 3×10^6 will exceed the column adsorption capacity. So when starting to explore the experimental conditions, if the RNA content of the sample is not clear, it is better to use a smaller sample processing volume, such as cells not exceeding $3\text{-}4\times10^6$ 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 and Buffer 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 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 residues of DNA during the extraction process (DNase digestion cannot achieve 100% residue free). Our EASYspin RNA Mini Kit extraction product, due to the use of our unique buffer system and genomic DNA clearance column technology, the vast majority of DNA has been cleared 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 up after DNase I treatment. Please contact us for specific operating instructions.
   5.4. Before rinsing with Buffer RW1, perform DNase I treatment directly on the adsorption column RA. Please contact us to request specific operating instructions (DNase Digestion Kit:RNK3401).
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 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 appearance of small dispersed flakes or disappearance of bands indicates severe degradation of the sample. But attention should be paid to distinguishing whether the extracted RNA sample itself degraded or extracted.
   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' products have a lower ratio 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 a high level of 2.1 to 2.2 purity). 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, the reading measured in an aqueous solution may be between 1.5-1.9, but 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: Before first use, please add the specified amount of anhydrous ethanol to the Buffer RW bottle and 70% ethanol bottle!

1. Cultivate cells

1.1. **Adhering cells:** do not require digestion, completely aspirate the culture liquid and directly add the recommended amount of Buffer RLT (see Appendix 1). Repeatedly blow and beat the cells for lysis (after lysis, directly follow step 3 of the operation); 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.

1.1.1. **Suspended cells:** Collect<10^7 suspended cells into a 1.5ml centrifuge tube.

1.2. 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 Buffer RLT and lead to a decrease in yield and purity.

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

1.4. Immediately proceed to step 3 of the Protocol

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 to fresh tissue, the tissue is thoroughly ground and homogenized using a glass or electric homogenizer.

2.1.1. **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 tissue buffer RLT. Shake vigorously for 20 seconds. For difficult to break 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. Transfer the supernatant to a new 1.5ml centrifuge tube.

2.2. Immediately proceed to step 3 of the operation.

3. Accurately estimate the volume of the lysate (supernatant) and add 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.
4. 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.
5. Add 700μl Buffer RW1, let it stand at room temperature for 30 seconds, centrifuge at 13000rpm for 30 seconds, and discard the waste liquid.
6. 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. Add 500μl Buffer RW and repeat.
7. 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.
8. 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 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 amount of culture medium (mL)	Available cell count
24 well culture plate	2	1.0	5×10^5
6-well culture plate	9.6	2.5	2.5×10^6
3.5cm culture dish	8	3.0	2.0×10^6
6cm culture dish	21	5.0	5.2×10^6
25cm plastic culture bottle	25	5.0	5.2×10^6
100ml glass culture bottle	33	10.0	7×10^6

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