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EZ spin plus Maxi Tissue/cell fast RNA extraction kit

Product Number: RNK4101

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	RNK4101 10 Preps
Buffer RLT Plus	RT	100 ml
Buffer RW1	RT	120 ml
Buffer RW	RT	25ml×2
RNase-free H_2O	RT	10 ml
70% ethanol	RT	15ml×2
Genomic DNA clearance column and collection tube	RT	10
RNase free adsorption column RA and collection tube	RT	10

Description

On the basis of our company's exclusive introduction of EASYspin phenol free and chloroform based rapid RNA extraction technology, we have 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 in 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 H20 elutes pure RNA from the silica matrix membrane.

Features

1. No toxic reagents such as phenol and chloroform are needed, and no steps such as ethanol precipitation are required.
2. Fast and simple, the operation of a single sample can generally be completed within 60 minutes.
3. The exclusive development of genomic DNA clearance column technology ensures effective clearance of gDNA residues, and the obtained RNA does not require DNase digestion and can be directly used for PCR, fluorescence quantitative PCR and other experiments.
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.

Application

Suitable for rapid extraction of total RNA from animal cells and easily lysed animal tissues, using unique genomic DNA clearance column technology to ensure effective removal of gDNA residues, without the need for DNase digestion. RNA can be directly used for PCR and fluorescence quantification of PCR.

Note

1. All centrifugation steps can be completed at room temperature using a centrifuge that can accommodate 50ml centrifuge tubes.
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 100mg will exceed the column processing capacity. COS cells have abundant RNA content, exceeding 6×10^7 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 6×10^7 and tissues not exceeding 200mg. In the future, the processing capacity will be increased or decreased based on the sample testing situation.
3. Buffer RLT Plus 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:

   Generally speaking, any total RNA extraction reagent cannot completely avoid trace residues of DNA 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 a relatively small impact on extremely small amounts of DNA residues in most RT-PCR amplification processes (which are generally not visible under electrophoretic EB staining UV lamps), If strict mRNA expression analysis, such as fluorescence quantitative PCR, is required, we recommend that when selecting templates and primers:

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

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

   4.3. 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.

   4.4. Before rinsing buffer RW1, perform DNase I treatment directly on the adsorption column RA. Please contact us to request specific operating instructions.
5. RNA purity and concentration detection:

   5.1. **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.

   5.2. **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.

   5.3. **Concentration:** Take a certain amount of RNA, 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)

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

1. Tissue cultured cells

   1.1. Collect<2×10^8 suspended cells into a suitable size centrifuge tube. For adherent cells, well plate culture can directly lyse them. Cell bottle culture should first be digested with trypsin and then collected by blowing.

1.2. Centrifuge 10000-1300×g for 20 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 Buffer RLT Plus, leading to a decrease in yield and purity.

1.3. Lightly flick the tube wall to completely loosen and resuspend the cell precipitate. Add 5ml (<10^8 cells) or 10ml (1×10^8-2×10^8 cells) of Buffer RLT Plus, blow and mix well, then vigorously shake by hand for 20 seconds to fully lyse.

1.4. Homogenization: (When dealing with very small cell volumes<2×10^6, it is generally not necessary, vortex oscillation for one minute to homogenize). Using a disposable 10ml syringe with a blunt needle (paired with a 0.9mm needle), vigorously tap the lysate more than 10 times or until satisfactory homogenization results are obtained (or electric homogenization for 60 seconds). This can cut DNA, reduce viscosity, prevent clogging of the column, and increase yield.

1.5. Add all the lysis mixture or homogenate mixture onto the DNA scavenging column (the scavenging column is placed in a collection tube).

1.6. Immediately proceed to step 3 of the protocol.

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

2.1. Electric homogenization: Quickly cut fresh tissue into small pieces with a dissecting knife, add 5ml (<250mg tissue) or 10ml (400-500mg tissue) of Buffer RLT Plus, and thoroughly homogenize for 1 minute.

2.2. Liquid nitrogen grinding+homogenization: After grinding the tissue into fine powder in liquid nitrogen, take an appropriate amount of tissue fine powder (250mg/500mg) and transfer it into a 50ml centrifuge tube containing 5ml/10ml Tissue Buffer RLT Plus. Shake vigorously by hand for 20 seconds to fully crack. Using a disposable 10 ml syringe with a blunt needle (paired with a 0.9mm needle), vigorously tap the lysate 10 times or until satisfactory homogenization results are obtained (or electric homogenization for 60 seconds). This can cut DNA, reduce viscosity, prevent clogging of the column, and increase yield.

2.3. Centrifuge 10000-1300×g of the lysate after homogenization for 5 minutes, precipitate any fragments or insoluble substances that may be difficult to lyse, and add all the supernatant of the lysate to the DNA clearance column (the clearance column is placed in a collection tube).

2.4. Immediately proceed to step 3 of the protocol.

3. Immediately centrifuge 10000-1300×g for 5 minutes 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. Use a micropipette to accurately estimate the volume of the filtrate (usually 5ml/10ml, the loss volume should be subtracted during filtration). 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, and do not centrifuge.

5. Add the mixture to an adsorption column RA and centrifuge 10000-1300×g for 3 minutes (ensuring that everything passes and there is no residual liquid on the membrane, otherwise increase the speed and time). Discard the waste liquid.

6. Add 10ml of Buffer RW1, let it stand at room temperature for 1 minute, centrifuge 12000×g for 3 minutes, and discard the waste liquid.

7. Add 10ml of Buffer RW (please check if anhydrous ethanol has been added first!), centrifuge 10000-1300×g for 1-2 minutes, and discard the waste liquid. Add 10ml Buffer RW and repeat.

8. Put the adsorption column RA back into the empty collection tube, centrifuge 13000×g for 5 minutes to dry the residual ethanol in the membrane matrix, use the nozzle to remove any residual ethanol between the inner ring pressure ring and the column wall, and air dry at room temperature or in an oven for a few minutes.

9. Take out the adsorption column RA and place it in an RNase free centrifuge tube. Add 500ul -1ml of RNase free H_2O to the middle of the adsorption membrane according to the expected RNA production. Leave at room temperature for 3 minutes and centrifuge at 12000×g for 2 minutes.

10. If the expected RNA production is>0.6mg, add 300-500μl of RNase free H_2O and repeat step 9. Combine the two eluents, or use the first eluent to add back to the adsorption column and repeat step once (if high RNA concentration is required).

The concentration of RNA elution solution after two washes is high, and the RNA production of the combined elution

solution after two washes is 15-30% higher than the former, but the concentration is lower. Users can choose according to their needs.