MEBEP BIOSCIENCE
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EASYspin Plus Bacterial Fast RNA Kit

Product Number: RNK4302

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°C water bath for a few minutes to restore clarity.
2. Inappropriate storage at low temperatures (4°C or -20°C) can cause solution precipitation, affecting the effectiveness of use. Therefore, transportation and storage are carried out at room temperature (15°C -25°C).
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.

Component

Component	Storage	RNK4302 50preps
TE (PH8.0)	RT	6 ml
Lysozyme	4°C	20 mg
Buffer RLT Plus	RT	25 ml
Buffer RW1	RT	40 ml
Buffer RW	RT	10ml
70% ethanol	RT	9ml RNase-free H_2O
RNase-free H_2O	RT	10 ml
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 EASYspin phenol free and chloroform RNA rapid extraction technology launched by our company, we have also independently developed the 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. 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 washes the pure RNA off the silica matrix membrane.

Feature

1. No toxic reagents such as phenol and chloroform are used, and no steps such as ethanol precipitation are required.
2. Fast and simple, the operation of a single sample can generally be completed within 30 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 2.0-2.2 and almost no DNA residue. It can be used for RT-PCR, Northern blot, and various experiments.

Applications

Suitable for rapid extraction of bacterial total RNA, 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 reverse transcription PCR and fluorescence quantification PCR.

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 13000 rpm.
2. The sample processing capacity should never exceed the processing capacity of the genomic adsorption column DA and RNA adsorption column RA, otherwise it may cause DNA residue or yield reduction. When starting to explore the experimental conditions, if the DNA/RNA content of the sample is unclear, it is better to use a smaller sample processing volume, and increase or decrease the processing volume in the future according to the sample testing situation.
3. Buffer RLT Plus 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. 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 Plus 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 the extremely small amount of DNA residue in most RT-PCR amplification processes (which is generally not visible under electrophoresis EB staining UV light observation), 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 cross the connections 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 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.
   4.4. Before rinsing buffer RW1, perform DNase I treatment directly on the adsorption column RA. Please contact us to request the specific operating manual (RNK3401).
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 bacteria being rRNA, very clear rRNA bands should be visible under UV after electrophoresis. The size of bacterial rRNAs is approximately 5 kb and 2 kb, which are equivalent to 26S and 13S rRNAs, respectively. The maximum rRNA brightness in bacterial 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 a reference indicator for measuring the degree of protein contamination. High quality RNA, OD260/OD280 reading (10mMTris, pH 7.5) between 2.1-2.2 (100% pure RNA ratio is generally around 2.2, which many companies cannot meet, so 1.9-2.0 is sufficient, but our product standards can generally reach 2.1-2.2). 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 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

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bottle!

2)To extract bacterial RNA, it is necessary to first prepare TE (10mM Tris HCl, 1mM EDTA) with added lysozyme or lysostaphin. Lysozyme or lysostaphin has been added to TE at a concentration of 1mg/ml.

1. Collect 1-2ml of bacterial solution (10^8-10^9 cells) by centrifugation into a 1.5ml centrifuge tube, remove the supernatant as much as possible, and ensure that the residual supernatant does not exceed 20l/100l TE is used (see step 2 below).
2. According to the type and quantity of cells, fully resuspend the cells in 100μl (5-10^8 cells)/200μl (5×10^8-7.5×10^8cells) TE (10 mM Tris HCl, 1 mM EDTA), with lysozyme or lysostaphin added at a concentration of 1mg/ml, or directly resuspend with TE. Use a clean gun to pick a small amount of lysozyme and add it.
3. Incubate at room temperature (15-25 °C) for 5 minutes/lysozyme, or at 37 °C for 15 minutes/lysostaphin to break the cell wall. Every 2 minutes, the vortex oscillates for 10 seconds to help break through the wall.
   Note: The difficulty of various bacteria breaking walls varies. Generally, the above conditions are sufficient for the use of Gram negative bacteria E. coli, and this step may even be omitted. However, some Gram positive bacteria such as B Subtilis needs to increase the concentration of lysozyme to 15mg/ml and incubate for 10 minutes to break the wall. If Staphylococcus aureus needs to be added with lysostaphin to 1mg/ml, incubate at 37°C for 15 minutes. In short, different bacterial types have varying degrees of difficulty in breaking walls. Some types of difficult to break walls require adjusting the enzyme type, working concentration, incubation temperature, and time according to the user's specific situation. In addition, methods such as glass bead impact, mechanical wall breaking, and protease K digestion can also be used in combination to help break walls.
4. Collect cells by brief centrifugation to the bottom of the tube and discard the supernatant. Vortex oscillation resuspend dispersed cells.
5. Add 500μl Buffer RLT Plus, blow and mix well, then vigorously shake by hand for 20 seconds to fully crack.
   Generally, after adding Buffer RLT Plus and thoroughly vortex blowing, no obvious clumps or insoluble substances should be seen. In rare cases, if there are obvious clumps or insoluble substances, the lysate can be centrifuged at 13000rpm for 3 minutes to precipitate the fragments or insoluble substances that cannot be lysed. The supernatant of the lysate should be transferred to a new centrifuge tube before proceeding to the next step.
6. Immediately add the lysate to a DNA clearance column (placed in a collection tube) and centrifuge at 13000 rpm for 30 seconds, retaining 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.
7. Use a micropipette to accurately estimate the volume of the filtrate (usually 500μl, the volume lost during filtration should be subtracted), 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.
8. Immediately add the mixture (less than 700μl 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.
9. Add 700μl Buffer RW1, let it stand at room temperature for 30 seconds, centrifuge at 13000rpm for 30 seconds, and discard the waste liquid.
10. 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.
11. 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.
12. 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 production. Leave at room temperature for 1 minute and centrifuge at 13000 rpm for 1 minute.
13. If the expected RNA production is>30g, add 30-50μl of RNase free water and repeat step 10. 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

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to their needs. Buffer RLT Plus. The maximum processing capacity does not exceed 10^7 cells.