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EASYspin Bacterial Fast RNA Kit

Product Number: RNK0801

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. Improper storage at low temperatures (4℃ or -20℃) can cause solution precipitation and affect 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	RNK0801 20Preps	RNK0802 50Preps
TE (PH8.0)	RT	6ml	6ml
lysozyme	4℃	20mg	20mg
Buffer RLT	RT	20 ml	50 ml
Buffer RW1	RT	15 ml	40 ml
Buffer RW	RT	5ml	10ml
RNase-free H_2O	RT	10 ml	10 ml
RNase free adsorption column RA and collection tube	RT	20	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 and centrifugation steps, Buffer RW1 and Buffer RW remove impurities such as cellular metabolites and proteins. Finally, low salt RNase free H_2O washes pure RNA off the silica matrix membrane

Features

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 minimal DNA residue, which can be used for RT-PCR, Northern blot, and various experiments.

Application

Suitable for rapid extraction of total bacterial RNA

Note

1. Before using it for the first time, please add the specified amount of ethanol to the Buffer RW bottle. After adding, please mark the ethanol added in a timely manner to avoid adding it multiple times!
2. **All centrifugation steps are completed at room temperature** using a traditional desktop centrifuge with a rotational speed of 13000rpm, such as Eppendorf5415℃ or a similar centrifuge.

3. Self prepared ethanol is required.
4. 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.**
5. To prevent RNase pollution, attention should be paid to the following aspects:
   5.1. Regularly replace with new gloves. Because the skin often carries bacteria, it may lead to RNase contamination
   5.2. Use RNase free plastic products and gun heads to avoid cross contamination.
   5.3. 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.
   5.4. Prepare the solution using water without RNase. (Add water to a clean glass bottle, add DEPC to the final concentration of 0.1% (w/v), leave at 37℃ overnight, and sterilize under high pressure.)
6. 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:
   6.1. Select primers that cross introns to pass through the connecting regions in mRNA, so that DNA cannot serve as a template for amplification reactions.
   6.2. Select primer pairs with different sizes of amplified products on genomic DNA and cDNA.
   6.3. Treat the RNA extract with DNaseI of RNase free. This reagent kit can also be used for RNA cleaning after DNaseI treatment. Please contact us for specific operating instructions.
   6.4. Before rinsing buffer RW1, perform DNaseI treatment directly on the adsorption column RA. Please contact us to request specific operating instructions.
7. RNA purity and concentration detection:
   7.1. Integrity: RNA can be detected by ordinary agarose gel electrophoresis (electrophoresis conditions: gel concentration 1.2%; 0.5xTBE 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 5kb and 2kb in size, equivalent to 28S and 18S 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.
   7.2. Purity: The OD260/OD280 ratio is an indicator of the degree of protein contamination. High quality RNA, OD260/0D280 reading (10mM Tris, 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 OD260/0D280 reading for the same RNA sample is between 1.8-2.1 in a 10mM Tris, pH 7.5 solution, and may be between 1.5-1.9 in an aqueous solution, this does not mean that the RNA is impure.
   7.3. 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 ratio n)×40

Protocol(Please read the precautions before the experiment)

Tip: 1) Before first use, please add the specified amount of anhydrous ethanol to the Buffer RW 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 20μl per 100μl of TE 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 (5×10^8-7.5×10^8 cells) TE (10mM Tris HCl, 1mM EDTA), where lysozyme or lysostaphin has been 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 lysozyme at room temperature (15-25℃) for 5 minutes, or lysostaphin at 37℃ for 15 minutes to break the cell wall. Vortex for 10 seconds every 2 minutes to assist in breaking the wall.

   Note:The difficulty of various bacteria breaking walls varies. Generally, the above conditions are sufficient for Gram negative bacteria to use, and this step may even be omitted. However, for some positive and difficult to break walls, it is necessary to increase the concentration of lysozyme or use methods such as lysostaphin, glass bead mechanical wall breaking, proteolytic enzyme K digestion, or combined use. The working concentration of enzymes, temperature, time, and selection of the correct method need to be adjusted according to the user's specific situation.
4. Add 350μl (if 100μl TE/enzyme is used on top) or 700μl (if 200μl TE/enzyme is used on top) of Buffer RLT, blow and mix well, then vigorously shake by hand for 20 seconds to fully lyse.

   Generally, after adding buffer RLT and thorough vortex blowing, no obvious clumps or insoluble substances should be seen. In rare cases, if there are obvious clumps or insoluble substances, the cracked product can be centrifuged at 13000rpm for 3 minutes to precipitate the fragments or insoluble substances that cannot be cracked. The entire supernatant of the cracked product should be transferred to a new centrifuge tube before proceeding to the next step.
5. Add 250μl 96-100% ethanol (previously added 100μl TE/350μl Buffer RLT tube) or 500μl 96-100% ethanol (previously added 200μl TE/700μl Buffer RLT tube), immediately blow and mix well.
6. Immediately add the mixture (less than 700μl each time, which can be added in two separate batches) to an adsorption column RA, centrifuge at 13000rpm for 60 seconds, and discard the waste liquid.
7. 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.
8. Add 500μl of Buffer RW (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid. Add 500μl Buffer RW and repeat.
9. Put the adsorption column RA back into the empty collection tube, centrifuge at 13000rpm for 2 minutes, and try to remove Buffer RW as much as possible to avoid residual ethanol in Buffer RW inhibiting downstream reactions.
10. 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 (heating in a water bath at 70-90℃ beforehand is more effective). Leave at room temperature for 1 minute and centrifuge at 12000 rpm for 1 minute.
11. If the expected RNA production is>30μg, 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 to their needs.