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EASYspin Yeast RNA Fast Kit

Product Number: RNK1001

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 reducing activity and facilitate transportation, Lyticase (2500U) is provided as a freeze-dried powder. After receiving it, it can be briefly centrifuged and dissolved in 0.25ml of sterilized water to prepare 10U/μl. Because repeated freeze-thaw cycles may reduce enzyme activity, immediately after dissolution, pack and freeze according to the amount used each time, and store at -20℃.
4. 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	RNK1001 50preps
Buffer SE	RT	30 ml
Lyticase 10U/μl	-20℃	2500U
Buffer RLT	RT	20 ml
Buffer RW1	RT	40 ml
Buffer RW	RT	10 ml
RNase-free H_2O	RT	10 ml
RNase free adsorption column RA and collection tube	RT	50

Description

Yeast cells undergo Lyticase treatment to remove cell walls, resulting in unique Buffer RLT/ β-Mercaptoethanol rapidly cleaves cells and inactivates cell RNA enzymes. After adjusting the binding conditions with ethanol, RNA selectively adsorbs onto the silica matrix membrane in a centrifuge column. 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 H2O 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 20 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.

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, β- Mercaptoethanol, water bath.
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. Regarding trace residues of DNA:

In general, 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, do not have a significant impact on the extremely small amount of DNA residues during 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:

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

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

5.3. 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.

5.4. Before rinsing Buffer RW1, perform DNaseI treatment directly on the adsorption column RA. Please contact us to request specific operating instructions.

6. RNA purity and concentration detection:

6.1. Integrity: RNA can be detected by ordinary agarose gel electrophoresis (electrophoresis conditions: gel concentration 1.2%; 0.5xTBE 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 RNA sizes are approximately 5kb and 2kb, equivalent to 28S and 18S rRNA, 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.

6.2. 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.

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: 1) Before first use, please add the specified amount of anhydrous ethanol to the Buffer RW bottle!

2) Before operation, add β-mercaptoethanol to the Buffer RLT until the final concentration is 1%. For example, **add 10μl of β-mercaptoethanol to 1ml of Buffer RLT.** It is best to use and configure this Buffer RLT as it is. The prepared Buffer RLT4℃ can be stored for one month.

3) Add 0.1% β-mercaptoethanol to the Buffer SE used for later use.

1. Cultivating cells with small amounts of yeast

1.1. Collect 1ml (approximately 10^7 cells) of yeast culture in logarithmic growth phase into a 1.5ml centrifuge tube, centrifuge at 12000 rpm for 30 seconds, and discard the supernatant as much as possible.

1.2. Add 100μl of Buffer SE **(confirm that β-mercaptoethanol has been added to the final concentration of 0.1%)**, gently blow and resuspend the cells thoroughly; Add about 50U Lyticase according to the amount of yeast, mix thoroughly upside down, and incubate at 37℃ for 15-30 minutes to digest the cell wall. Invert several times in the middle to aid

digestion.

If the wall breaking effect is not good and leads to low RNA production, the dosage of Lyticase can be increased to increase the enzyme working concentration, and the digestion time can be extended to improve the effect. Yeasts that are not suitable for Lyticase digestion can choose Zymolase or other methods such as glass bead vortex and repeated freeze-thaw.

1.3. Add 350μl Buffer RLT (confirm that β-mercaptoethanol has been added to the final concentration of 1%), blow and mix well, then vigorously shake by hand for 20 seconds to fully crack.

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.

1.4. Add 350μl of 96-100% ethanol, immediately blow and mix well, do not centrifuge.

1.5. Follow step 3 of the operation.

2. Medium amount yeast culture of cells

2.1. Collect 2-3ml (approximately 3×10^7 cells) of yeast culture in logarithmic growth phase into a 1.5ml centrifuge tube (if the volume exceeds 1.5ml, the cells can be collected twice in the same centrifuge tube), centrifuge at 12000 rpm for 30 seconds, and discard the supernatant as much as possible.

2.2. Add 600μl of Buffer SE (confirm that β-mercaptoethanol has been added to the final concentration of 0.1%), gently blow and resuspend the cells thoroughly; Add approximately 100-150U of Lyticase according to the amount of yeast, mix thoroughly upside down, and incubate at 37℃ for 15-30 minutes to digest the cell wall. Invert several times in the middle to aid digestion.

If the wall breaking effect is not good and leads to low RNA production, the dosage of Lyticase can be increased to increase the enzyme working concentration, and the digestion time can be extended to improve the effect. Yeasts that are not suitable for Lyticase digestion can choose Zymolase or other methods such as glass bead vortex and repeated freeze-thaw.

2.3. Centrifuge at 13000rpm for 1 minute and discard the supernatant as much as possible.

2.4. Add 350μl Buffer RLT (confirm that β-mercaptoethanol has been added to the final concentration of 1%), blow and mix well, then vigorously shake by hand for 20 seconds to fully crack.

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.

2.5. Add an equal volume of 70% ethanol (prepared with DEPC water, about 350μl) and immediately blow and mix well without centrifugation.

2.6. Follow step 3 of the operation.

3. Add the mixture to an adsorption column RA, centrifuge at 13000rpm for 30-60 seconds (with the adsorption column placed in the collection tube), and discard the waste liquid.

4. 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.

5. 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.

6. 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.

7. Take out the adsorption column RA and place it in an RNase free centrifuge tube. Add 30-50μl of RNase free water (pre heated in a 70-80℃ water bath) to the middle of the adsorption membrane according to the expected RNA production. Leave at room temperature for 1 minute and centrifuge at 12000rpm for 1 minute.
8. If the expected RNA production is>30μg, add 30-50μl of RNase free water and repeat step 7 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.