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EASYspin Plus Plant Fast RNA

Product Number: RNK3802

Shipping and Storage

1. 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℃).
2. 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	RNK3802 50Preps
Buffer RLT	RT	50 ml
Buffer CLB（presenter）	RT	8 ml
Buffer RLT Plus	RT	25 ml
Buffer RW1	RT	40 ml
Buffer RW	RT	10 ml
RNase-free H_2O	RT	10 ml
PLANTaid	RT	5 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 our company's exclusive introduction of EASYspin phenol free and chloroform based rapid RNA extraction technology, we have also independently developed the genome DNA clearance column technology, which can effectively remove gDNA residues. The obtained RNA generally does not require DNase digestion and can be used for reverse transcription PCR, fluorescence quantitative PCR and other experiments. The unique Buffer RLT rapidly cleaves cells and inactivates cellular RNA enzymes. The plant RNA co extractant PLANTaid helps to bind to polysaccharide polyphenols and remove them by centrifugation. Then, the mixture is lysed and the RNA binding is regulated by ethanol to adsorb onto the genomic DNA scavenging column. The RNA is selectively washed and filtered, and the residual DNA adsorbed on the genomic DNA scavenging column cannot be washed away. The column is discarded together to remove the DNA. 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 H2O washes the pure RNA off the silica matrix membrane.

Features

1. No toxic reagents such as phenol and chloroform are used, and no steps such as ethanol precipitation are required.
2. Simplicity, single sample operation can generally be completed within 25 minutes, making it the simplest and fastest reagent kit in the world.
3. The unique plant RNA extractant PLANTaid can effectively bind polysaccharides and polyphenols, improving clearance efficiency.
4. The independently developed genomic DNA clearance column technology can effectively remove gDNA residues, and the obtained RNA generally does not require DNase digestion and can be used for experiments such as reverse transcription PCR and fluorescence quantitative PCR.
5. The world's leading adaptability is extremely extensive, and it can extract hundreds of samples that have failed to be extracted

by domestic and foreign reagent kits, including cotton, roses, Arabidopsis, poplar, and so on.

6. Multiple column washes ensure high purity, with a typical OD260/OD280 ratio of 2.1~2.2 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 plant tissue cells, unique genomic DNA clearance column technology can effectively remove visible gDNA residues on electrophoresis. RNA can be used for reverse transcription PCR, fluorescence quantitative PCR, etc.

Note

1. All centrifugation steps can be completed at room temperature (centrifugation at 4℃ is also possible), using a traditional desktop centrifuge with a speed of 13000 rpm, such as Eppendorf 5415C or a similar centrifuge.
2. Self prepared ethanol and mortar (optional) are required.
3. The sample processing capacity should never exceed the genomic clearance column DA and RNA adsorption column RA processing capacity, 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, a smaller sample processing amount can be used. In the future, the processing amount can be increased or decreased according to the sample testing situation.
4. Buffer RLT, RLT Plus, and Buffer RW1 contain irritating compounds. Wear latex gloves 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:

   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 Plus RNA extraction product adopts our unique buffer system and genomic DNA clearance column technology, and the vast majority of DNA has been cleared without 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 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 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.

   5.4. Before rinsing buffer RW1, perform DNase I column digestion directly on the adsorption column RA. Can purchase DNase Digestion Kit (RNK3401)
6. Please read Supplementary Note 2 carefully. If the Buffer CLB works well, you can contact us to order the Buffer CLB separately. In the future, you can directly order EASYspin Plus complex Plant RNA Kit (RNK5301).

Protocol(Please read the notes before the experiment)

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

1. **Direct grinding method (recommended for extracting simple plant samples, but liquid nitrogen grinding method can also be used for simple samples):**

   1.1. After weighing fresh plant tissue, quickly cut 100mg-200mg into small pieces and place them in a mortar (frozen or liquid nitrogen stored samples can be directly weighed and then 100mg-200mg can be placed in a mortar). Add 10 volumes (1ml) of RLT and 1 volume (100μl) of PLANTaid and grind them thoroughly at room temperature to form a homogenate. It is important to grind quickly to allow the tissue and Buffer RLT to come into full contact immediately to inhibit RNA enzyme activity.

   Note: PLANTAid is an essential component in difficult samples for extracting pigments from secondary

metabolites of polysaccharides and polyphenols.

1.2. Transfer the lysate into a centrifuge tube, vigorously shake and oscillate for 15 seconds, centrifuge at 13000 rpm for 5-10 minutes, and precipitate the fragments that cannot be lysed and the PLANTAid bound with polysaccharides and polyphenols.

1.3. Take 480μl of lysate supernatant (more supernatant can be taken without exceeding the capacity of the genomic DNA scavenging column, which can increase yield) and transfer it to a new centrifuge tube. Add half the volume of anhydrous ethanol (0.5 volume) to the supernatant. Precipitation may occur at this time, but it does not affect the extraction process. Immediately blow and mix well without centrifugation.

1.4. Immediately follow step 3 of the operation steps.

2. Liquid nitrogen grinding method (recommended for extracting complex and easily degradable samples):

2.1. Take 500μl Buffer RLT and transfer it into a 1.5ml centrifuge tube. Add 50μl PLANTAID and mix well for later use.

2.2. After grinding an appropriate amount of plant tissue into fine powder in liquid nitrogen, take 50mg-100mg of fine powder and transfer it into the centrifuge tube containing RLT and LANTaid mentioned above. Immediately shake vigorously by hand for 20 seconds to fully decompose.

2.3. Blow and mix with a suction head to aid in cracking or vigorous vortex shaking until satisfactory homogenization results are obtained (or electric homogenization for 30 seconds), which can shear DNA, reduce viscosity, and increase yield.

2.4. Centrifuge the lysate at 13000 rpm for 5-10 minutes, and precipitate the non lysable fragments and PLANTAID bound with polysaccharides and polyphenols.

2.5. Take the supernatant of the lysate (more supernatant can be taken without exceeding the capacity of the genomic DNA scavenging column, which can increase yield) and transfer it to a new centrifuge tube. Add half the volume of anhydrous ethanol (0.5 volume) to the supernatant. Precipitation may occur at this time, but it does not affect the extraction process. Immediately blow and mix well without centrifugation.

2.6. Immediately follow step 3 of the operation steps.

Note: Users of the above liquid nitrogen grinding method can double the processing as needed to increase production. That is to use 1ml of Buffer RLT, 100μl of LANTaid, and 100mg-200mg of samples.

3. Add the mixture (less than 720μl each time, which can be added in two separate batches) to a genome clearance column. Centrifuge at 13000 rpm for 2 minutes and discard the waste liquid.

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. Place the genomic DNA clearance column in a clean 2ml centrifuge tube (without RNA free or DEPC treatment, usually a clean new centrifuge tube is sufficient. Alternatively, use a new clean collection tube that comes with the RNA adsorption column), add 500μl Buffer RLT Plus to the genomic DNA clearance column, centrifuge at 13000 rpm for 30 seconds, collect the filtrate (RNA in the filtrate), use a micropipette to accurately estimate the volume of the filtrate (usually around 450-500μl, the volume lost during filtration should be subtracted), and add 0.5 times the volume of anhydrous ethanol. Precipitation may occur at this time, but it does not affect the extraction process. Immediately blow and mix, and do not centrifuge.

5. 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 2 minutes (the adsorption column is placed in a collection tube), and discard the waste liquid.

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.

6. Add 700μl Buffer RW1, let it stand at room temperature for 1 minute, centrifuge at 13000rpm for 30 seconds, and discard the waste liquid.

7. 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.

8. 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.

9. 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 yield (heating in a 70-90 ℃ water bath beforehand can increase yield). Leave at room temperature for 1 minute and centrifuge at 12000 rpm for 1 minute.

10. If the expected RNA production is>30ug, add 30-50μl of RNase free water and repeat step 9. Combine the two washes, or use the first wash 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.

Supplementary note 1:

The general plant species are very c omplex, and the effectiveness of using this reagent kit varies for samples of different plant species and parts. Some samples may significantly reduce production while undergoing genomic DNA clearance columns to remove residual DNA. In this case, it is recommended that the customer try to extract the EASYspin RNA Plant Mini Kit (RNK0902) following the steps. Omit the steps for genomic DNA clearance columns, Compared to RNK3802.RNK0902 lacks one genomic DNA clearance column step, which may in some cases increase yield. If RNK0902 increases production while also increasing DNA residue, customers can add a DNA enzyme column digestion step RNK3401 according to experimental needs to remove residual DNA or use traditional DNA enzyme digestion to remove DNA residue.

Supplementary note 2:

Regarding the situation where the extraction of plant samples with particularly complex and difficult extraction fails or the yield is low:

For some particularly complex plant samples extraction, such as rice seeds, grape fruits, indigo fruit fruits, lily bulbs, potato tubers, etc., RNK3802's Buffer RLT cannot be extracted, so (RNK5301)-EASYspin Plus complex Plant RNA Kit needs to be selected. Some samples have lower yields and can also be attempted. RNK5301 adopts the powerful Buffer CLB option, which can extract complex samples or significantly increase production in many cases (please refer to the RNK5301 manual for details). This reagent kit currently comes with 8ml of Buffer CLB as a gift. You can test the effectiveness of Buffer CLB.

Appendix 1

The EASYspin RNA Plant Mini Kit (RNK0902) and EASYspin Plus Plant Fast RNA (RNK3802) operations are identical, except that steps 3 and 4 are omitted, and step 5 is directly followed after steps 1 and 2.

Appendix 2

EASYspin Plus Plant Fast RNA - (RNK3802) Using Buffer CLB Operation Steps

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

2) Take 1ml of Buffer CLB into a centrifuge tube (if there is precipitation or precipitation of CLB, it should be dissolved again in a 65°C water bath), and add 5% to the Buffer CLB β-Mercaptoethanol (1ml Buffer CLB+50μl) β-Mercaptoethanol. Invert and mix thoroughly before preheating in a 65°C water bath.

1. Grind fresh or -70°C frozen materials into fine powder in liquid nitrogen.
2. Transfer 100mg-200mg of fine powder (100mg-150mg can be added to samples with low moisture, such as seeds and leaves, and more can be added to samples with high moisture, such as watermelon) to a preheated Buffer CLB (already containing β-mercaptoethanol) centrifuge tube. Immediately vigorously vortex for 30-60 seconds or use a suction head to blow and mix, and then put it back into a 65°C water bath for a short time (5-10 minutes, slightly longer for 10 minutes, the yield may increase). Occasionally invert 1-2 times in the middle to help with cracking.

   β-mercaptoethanol is a key component of Buffer CLB, and the final concentration can be increased to 10-20% if necessary.
3. After shaking and mixing, centrifuge at 13000rpm for 10 minutes at room temperature.
4. Take the supernatant of the lysate (more supernatant can be taken without exceeding the capacity of the genomic DNA

scavenging column, which can increase yield) and transfer it to a new centrifuge tube. Add half the volume of anhydrous ethanol (0.5 volume) to the supernatant. Precipitation may occur at this time, but it does not affect the extraction process. Immediately blow and mix well without centrifugation.

If there is floating material on the surface of the upper clear, use a suction head to pick up the liquid below.

5. Immediately follow step 3 of the operation steps.