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EASYspin Plus Plant RNA Max Kit

Product Number: RNK4201

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.

Component

Component	Storage	RNK4201 10preps
Buffer RLT	RT	100 ml
Buffer RLT Plus	RT	50 ml
Buffer RW1	RT	120 ml
Buffer RW	RT	25 ml×2
RNase-free H_2O	RT	10 ml
PLANTaid	4℃	10 ml×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 the genome DNA clearance column technology, which can effectively remove gDNA residues. The obtained RNA generally does not require DNase digestion and can be directly used in PCR, fluorescence quantitative PCR and other experiments. Unique Buffer RLT/ β- Mercaptoethanol rapidly cleaves cells and inactivates cell RNA enzymes. Plant RNA co extractant PLANTaid helps to bind to polysaccharide polyphenols and remove them by centrifugation. Then, the mixture is cleaved using ethanol to regulate RNA binding and adsorption onto the genomic DNA clearance column. The genomic DNA is cleared and RNA is selectively eluted and filtered. 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 are used to remove impurities such as cell metabolites and proteins. Finally, the pure RNA is eluted from the silica matrix membrane with low salt RNase free H2O.

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 60 minutes.
4. The unique plant RNA extractant can effectively bind polysaccharides and polyphenols, improving the clearance effect.
5. 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.

Applications

Suitable for rapid extraction of total RNA from plant tissue cells, the use of unique genomic DNA clearance column technology can effectively remove DNA residues, generally without the need for DNase digestion. RNA can be directly used for PCR and fluorescence quantitative PCR.

Note

1. All centrifugation steps can be completed at room temperature using a centrifuge that can accommodate 50ml centrifuge tubes.
2. Self provided ethanol, disposable syringe (optional), and mortar are required.
3. Buffer RLT, Buffer RLT Plus, and Buffer RW1 contain irritating compounds. When operating, wear latex gloves to avoid contact with skin, eyes, and clothing. If it gets on the skin or eyes, it is necessary to rinse with a large amount of water or physiological saline.
4. The adequacy of plant tissue lysis directly affects the quality and yield of RNA extraction. The Buffer RLT provided in this kit is mainly composed of guanidine isothiocyanate, which is suitable for the lysis of most plant tissues. However, for some tissues (such as the milky white endosperm of corn) or filamentous fungi, due to the special secondary metabolites, guanidine isothiocyanate causes the sample to solidify, resulting in the inability to extract RNA. In this case, we can request another Buffer RLC to solve this problem.
5. 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, do not have a significant 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 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%; 0.5×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 RNA sizes are approximately 5kb and 2kb, equivalent to 28S and 18S RNA, 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: Before first use, please add the specified amount of anhydrous ethanol to the Buffer RW bottle!

1. **Direct grinding method (recommended):**

1.1. After weighing fresh plant tissues, take 1-2g and quickly cut them into small pieces and place them in a mortar (frozen or liquid nitrogen stored samples can be directly weighed and then take 1-2g and place them in a mortar). Add **10 volumes (10ml)** of Buffer RLT and **1 volume (1ml)** of PLANTaid to fully grind into a homogenate at room temperature. It is important to grind quickly to allow the tissues and Buffer RLT to come into full contact immediately to inhibit RNA enzyme activity.

Note: PLANTaid is an essential component for extracting samples with abundant polysaccharide and polyphenol content. Extracting ordinary plant tissue without the addition of PLANTaid may increase RNA production slightly.

1.2. Transfer the lysate into a centrifuge tube, vigorously shake and oscillate for 15 seconds, centrifuge at 10000-13000×g for 10 minutes (if the centrifuge speed is low, the centrifugation time can be appropriately extended), precipitate the fragments that cannot be lysed and the PLANTaid bound with polysaccharides and polyphenols, carefully take the **supernatant of the lysate (volume needs to be calculated)** and transfer it to a new centrifuge tube.

1.3. 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 shake vigorously and mix well without centrifugation.

1.4. Immediately proceed to step 3 of the operation.

2. **Liquid nitrogen grinding method:**

2.1. Take 10ml of Buffer RLT and transfer it into a 50ml centrifuge tube. Add 1ml of PLANTaid and mix well for later use.

2.2. After grinding an appropriate amount of plant tissue into fine powder in liquid nitrogen, take 1-2g of the fine powder and transfer it into the centrifuge tube containing Buffer RLT and PLANTaid mentioned above. Immediately shake vigorously by hand for 20 seconds to fully decompose.

Heating at 56℃ for 1-3 minutes helps to break down plants, but plants with high starch content cannot be heated because the increased temperature may cause starch swelling.

2.3. Using a disposable 5ml syringe with a blunt needle (paired with a 0.9mm needle), the lysate can be aspirated 10 times or until satisfactory homogenization results are obtained (or electric homogenization for 30 seconds), which can cut DNA, reduce viscosity, and increase yield.

2.4. Centrifuge the lysate at 10000-13000×g for 10 minutes, precipitate the non lysable fragments and PLANTaid bound with polysaccharides and polyphenols, and transfer all the supernatant of the lysate to a new centrifuge tube.

2.5. Accurately estimate the volume of the lysate (supernatant) and add **0.5 volume** of anhydrous ethanol. At this time, precipitation may occur but it does not affect the extraction process. Immediately shake vigorously and mix well without centrifugation.

2.6. Immediately proceed to step 3 of the operation.

3. Add the mixture to a genome clearance column (adsorption column placed in collection tube) and centrifuge for 5 minutes at 10000-13000×g (ensure that everything passes through and there is no residual liquid on the membrane, otherwise increase the speed and time), and discard the waste liquid.

4. Place the genomic DNA clearance column in a clean 50ml centrifuge tube (without RNA free or DEPC treatment, usually a clean new centrifuge tube is sufficient. Alternatively, use a new clean collection tube that matches the RNA adsorption column), add 5ml Buffer RLT Plus to the genomic DNA clearance column, centrifuge at 13000rpm for 2 minutes, collect the filtrate (RNA is in the filtrate), and use a micropipette to accurately estimate the volume of the filtrate (usually around 4-5ml, the volume lost during filtration should be subtracted). Add 0.5 times the volume of anhydrous ethanol. Precipitation may occur at this time, but it does not affect the extraction process. Blow and mix immediately without centrifugation.

5. Add the mixture to an RNA adsorption column RA (the adsorption column is placed in a collection tube) and centrifuge for 5 minutes at 10000-13000×g (ensure that everything passes through and there is no residual liquid on the membrane, otherwise the speed and time should be increased), and discard the waste liquid.

6. Add 6ml 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 at 10000-13000×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 at 13000×g for 5 minutes to dry the residual ethanol in the membrane matrix. Use the nozzle to remove any remaining ethanol between the inner ring pressure ring and the column wall, and let it 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 500μl-1ml of RNase free H_2O to the middle of the adsorption membrane according to the expected RNA production (better heating effect in a 70-90℃ water bath beforehand), 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.