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EASYspin Plus complex Plant RNA Kit

Product Number: RNK5301

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 air, each solution should be covered tightly in a timely manner after use

Components

Component	Storage	RNK5301 50Preps
Buffer CLB	RT	50 ml
Buffer RLT Plus	RT	25 ml
Buffer RW1	RT	40 ml
Buffer RW	RT	10 ml
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 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 CLB and Buffer RLT Plus rapidly lyse cells and inactivate cell RNA enzymes. Centrifuge precipitation is used to remove polysaccharides, polyphenols, and secondary metabolites. Then, the mixture is lysed and ethanol is used to regulate RNA binding and adsorption onto the genomic DNA clearance column. RNA is selectively washed and filtered, and the residual DNA adsorbed on the genomic DNA clearance column cannot be washed off. The column is discarded together to remove 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 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.
4. The world's leading adaptability is extremely extensive, and it can extract hundreds of failed samples from complex Chinese herbs such as dendrobium/salvia miltiorrhiza/snow lotus/ginseng, complex starch seeds such as rice/wheat/corn seeds, complex fruits such as grapes/blueberries/strawberries/watermelon fruits, complex stress resistant plants such as holly/pine needles/sea buckthorn/Populus euphratica, complex flowers such as roses/plums/peonies, complex polysaccharide plants such as seaweed/cactus/aloe vera, lily bulbs/rice seeds, etc. The EASYspin Plus complex Plant RNA Kit has published over 150

articles. For a detailed sample list, please refer to the company's homepage product introduction or contact us to request over 150 original published articles.

5. 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, second-generation sequencing, 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 provided β- Mercaptoethanol, ethanol, mortar (optional).
3. The sample processing capacity should never exceed the processing capacity of the genome clearance 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, 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 CLB, 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. Before purchasing the DNase Digestion Kit (RNK3401), you can first request specific operating instructions.

Protocol

Tips: 1) Before 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 CLB+50μl) β-Mercaptoethanol. Invert and mix thoroughly before preheating in a 65°C water bath. Multiple samples are prepared by scaling up in proportion.

1. Direct grinding method (recommended for laboratory conditions without liquid nitrogen or for soft and easily ground plant samples):

1.1. After weighing fresh plant tissue or frozen preservation samples, take 100mg-200mg (100mg-150mg can be added to samples with low moisture content such as leaves and seeds, and more can be added to samples with high moisture content such as strawberries and watermelon fruits). Quickly cut them into small pieces and place them in a mortar, then

add 1ml of CLB (already added) β-Mercaptoethanol should be thoroughly ground into a homogenate at room temperature, and it should be quickly ground to allow the tissue and Buffer CLB to immediately come into full contact to inhibit RNA enzyme activity.

β-Mercaptoethanol is a key component of Buffer CLB, and the final concentration can be increased to 10-20% if necessary.

If the plant is particularly complex, you can try adding PVP40 to the lysis solution until the final concentration is 2%.

1.2. Transfer the lysate into a centrifuge tube, immediately vigorously shake for 15 seconds, and briefly place it back in a 65 ° C water bath (5-10 minutes), occasionally inverting 1-2 times in the middle to aid in the lysis. Centrifuge at 13000rpm for 10 minutes to precipitate fragments that cannot be cracked.

1.3. 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.

1.4. Immediately follow step 3 of the operation steps.

2. Liquid nitrogen grinding method (widely applicable, recommended for extracting complex, difficult to break, and easily degradable samples):

2.1. Grind fresh or -70°C frozen materials into fine powder in liquid nitrogen.

2.2. Transfer 100mg-200mg of fine powder (100mg-150mg can be added to samples with low moisture content such as leaves and seeds, and more can be added to samples with high moisture content such as strawberries and watermelon fruits) to a preheated Buffer CLB (already containing mercaptoethanol) centrifuge tube. Immediately vortex vigorously for 30-60 seconds or use a suction head to blow and mix until satisfactory homogenization results are obtained (or electric homogenization for 30 seconds), which can shear DNA, reduce viscosity, and increase yield.

2.3. Put it back into a 65°C water bath for a short time (5-10 minutes), occasionally inverting it 1-2 times in the middle to aid in cracking.

2.4. Centrifuge the lysate at 13000 rpm for 10 minutes and precipitate the non lysable fragments.

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

2.6. Immediately follow step 3 of the operation steps.

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.

Appendix 1:DNA Enzyme Column Digestion (please refer to the RNK3401- DNase Digestion Kit manual for details)

1. Follow the RNK5301 operation steps listed earlier until step 5 is completed.
2. Take 45µl DNase I buffer and 5µl RNase free DNase I and gently blow them in a centrifuge tube to mix well to form a working solution (when processing multiple centrifuge columns, prepare the working solution by scaling up according to the ratio).
3. Add 350µl Buffer RW1 to the adsorption column RA, centrifuge at 12000 rpm for 30 seconds, discard the waste liquid, and place the adsorption column in the recovery header.
4. Add 50µl of DNase I working solution to the center of the adsorption column RA and let it stand at room temperature (20 ℃ -30℃) for 15 minutes.

   Pay attention to directly immerse the working liquid droplets in the center of the membrane and make full contact with the membrane around the membrane. Do not let the working liquid droplets hang on the O-shaped gasket or centrifugal column wall, or hang on the gasket without sufficient contact with the membrane.
5. Add 350µl Buffer RW1 to the adsorption column RA, centrifuge at 12000 rpm for 30-60 seconds, discard the waste liquid, and place the adsorption column in the recovery manifold.
6. Continue with step 7 and complete all subsequent steps.

Appendix 2: Solutions for Samples with Low RNA Content or Complex RNA Production

The sample processing capacity can be increased to 300-500mg/2ml Buffer CLB. After passing through two genomic DNA clearance columns in the supernatant, the eluted RNA can be combined into one RNA adsorption column, greatly increasing the RNA concentration.