MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

EASYspin RNA Plant Mini Kit

Product Number: RNK0902

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 the volatilization, oxidation, and pH changes of reagents exposed to air for a long time, each solution should be covered tightly in a timely manner after use.

Components

Component	Storage	RNK0902 50 Preps
Buffer RLT	4°C	50 ml
Buffer RW1	RT	40 ml
Buffer RW	RT	10 ml
RNase-free H_2O	RT	10 ml
PLANTaid	RT	5 ml
RNase free adsorption column RA and collection tube	RT	50

Description

The unique Buffer RLT rapidly cleaves cells and inactivates cell RNA enzymes. The plant RNA co extractant PLANTaid helps to bind to polysaccharide polyphenols and remove them by centrifugation. Then, 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 centrifugation steps, Buffer RW1 and Buffer RW remove impurities such as cell metabolites and proteins, Finally, low salt RNase free H_2O elutes pure RNA from the silicon substrate 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 world's advanced 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. For a detailed sample list, please refer to the product introduction on the company homepage.
5. Multiple column washes ensure high purity, with a typical OD260/OD280 ratio of up to 2.1-2.2 and minimal DNA residue. It can be used for RT-PCR, Northern blot, and various experiments.

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 13000 rpm.
2. Self prepared ethanol and mortar (optional) are required.
3. Buffer RLT 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.
4. 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 removed the vast majority of DNA residues. In most RT-PCR amplification processes, the impact of extremely small amounts of DNA residues is not significant, If strict mRNA expression analysis, such as fluorescence quantitative PCR, is required, we recommend that when selecting templates and primers:

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

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

4.3. 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.

4.4. Before rinsing buffer RW1, perform DNase I column digestion directly on the adsorption column RA. Before purchasing the DNase Digestion Kit (Product Number: RNK3401), you can first request specific operating instructions.

5. There are two options for extracting residual DNA from complex plant samples:

5.1. Before rinsing buffer RW1, perform DNase I column digestion directly on the adsorption column RA. Before purchasing the DNase Digestion Kit (Product Number: RNK3401), you can first request specific operating instructions.

5.2. When extracting some complex plant samples, there may be a lot of DNA residue. You can try our company's RNK3802: EASYspin Plus Plant Fast RNA. On the basis of RNK0902, RNK3802 has independently developed the genome DNA clearance column technology, which can effectively remove DNA residues. In most cases, DNA residues can be cleared until they are invisible under UV observation.

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

Some particularly complex plant samples, such as rice seeds, grape fruits, indigo fruit fruits, lily bulbs, potato tubers, etc., cannot be extracted using RNK0902: Buffer RLT, so RNK5301: EASYspin Plus complex Plant RNA Kit needs to be selected. Some samples have lower yields, but RNK5301: EASYspin Plus complex Plant RNA Kit can also be attempted. RNK5301 is equipped with a powerful Buffer RLTCLB option, which can extract complex samples or significantly increase production in many cases (please refer to the RNK5301 manual for details).

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 100mg-200mg 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 100mg-200mg and place them in a mortar). Add 10 volumes (1ml) of Buffer RLT and 1 volume (100μl) of PLANTAID and grind them thoroughly at room temperature to form a homogenate. It is important to quickly grind and allow the tissues 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 15s, 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 or all supernatants can be taken without exceeding the RNA adsorption capacity, which can increase yield) and transfer 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 RNA adsorption capacity, 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: The above liquid nitrogen grinding method allows users to double the processing as needed, which can 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 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.
4. Add 700μl Buffer RW1, let it stand at room temperature for 1 minute, centrifuge at 13000rpm for 30 seconds, and discard the waste liquid.
5. Add 500μl of Buffer RW (please check if anhydrous ethanol has been added first!), centrifuge at 13000rpm 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 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.
7. 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.
8. If the expected RNA production is>30ug, add 30-50μl of RNase free water and repeat step 7. 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 RNA concentration in the RNA elution solution after two washes is high, and the RNA production in 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 RNK0902 operation steps listed earlier until step 3 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 proportionally).
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 12000rpm for 30-60 seconds, discard the waste liquid, and

place the adsorption column in the recovery manifold.

6. Follow step 5 to complete the subsequent steps.