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EASYspin Universal Plant Fast RNA Kit

Product Number: RNK5201

Shipping and Storage

1. Improper storage of room temperature components at low temperatures (4℃ or-20℃) can cause solution precipitation and affect 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	RNK5201 50Preps
Buffer RPA	RT	50 ml
Buffer RW1	RT	40 ml
Buffer RW	RT	10 ml
RNase-free H_2O	RT	10 ml
DNase Buffer	-20℃	1.25 ml×2
RNase free DNase I	-20℃	0.25 ml
RNase free adsorption column RA and collection tube	RT	50

Description

The unique Buffer RPA rapidly cleaves cells and inactivates cellular RNA enzymes. After adjusting the binding conditions with ethanol, RNA selectively adsorbs onto the silica matrix membrane in a highly dissociated salt state. DNase directly digests residual DNA on the column, and then 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 washes pure RNA off the silica matrix membrane.

Features

1. Completely do not use toxic substances β-Mercaptoethanol/phenol/chloroform does not require steps such as ethanol precipitation.
2. Simplicity, single sample operation can generally be completed within 40 minutes, making it the simplest and fastest reagent kit in the world.
3. The RNA obtained through DNase I column digestion without residual DNase can be directly used for reverse transcription fluorescence quantitative PCR, second-generation sequencing, chip, RACE and other experiments.
4. World leading, it is the most widely adaptable reagent kit among similar products, which can extract plants including rice, corn, wheat, Arabidopsis, tomato, tobacco, and general polysaccharides and polyphenols such as cotton and holly.
5. Multiple column washes ensure high purity, with a typical OD260/OD280 ratio of 2.0-2.2 and no DNA residue. It can be directly used for fluorescence quantitative PCR, RT-PCR, chips, second-generation sequencing, Northern blot and other 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 RPA 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.

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):**
   1.1. After weighing fresh plant tissue, quickly cut 100-200mg into small pieces and place them in a mortar (frozen or liquid nitrogen stored samples can be directly weighed and then 100-200mg can be placed in a mortar). Add 1ml of Buffer RPA and grind thoroughly at room temperature to form a homogenate. It is important to grind quickly to allow the tissue and Buffer RPA to come into full contact immediately to inhibit RNA enzyme activity.
   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.
   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:**
   2.1. Take 500μl of Buffer RPA and transfer it into a 1.5ml centrifuge tube.
   2.2. After grinding an appropriate amount of plant tissue into fine powder in liquid nitrogen, take 50-100mg of fine powder and transfer it into the centrifuge tube containing RPA 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.
   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: Users of the above liquid nitrogen grinding method can double the processing as needed to increase production. That is to use 1ml of Buffer RPA and 100-200mg of sample.
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 350μl Buffer RW1, let it stand at room temperature for 1 minute, centrifuge at 13000rpm for 30 seconds, and discard the waste liquid.
5. Preparation of DNase I working solution: Take 45μl of DNase I buffer and 5μl of RNase free DNase I and gently blow them in a centrifuge tube to mix well to form the working solution (when processing multiple centrifuge columns, prepare the working solution by scaling up in proportion).
6. 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 drop the working liquid onto the center of the membrane, and do not let the working liquid drop onto the O-ring or centrifuge column wall.
7. 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.
8. 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.

9. 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.
10. 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.
11. If the expected RNA production is>30ug, add 30-50μl of RNase free water and repeat step 10. 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.

Note: If fluorescence quantitative PCR is not required and only regular reverse transcription is performed to clone gene fragments, the digestion step on the DNA enzyme column can be omitted. Specifically, in step 4, "add 350μl Buffer RW1" should be changed to "add 700μl Buffer RW1", and steps 5, 6, and 7 should be omitted.