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

EASYspin Plant microRNA Fast Kit

Product Number: RNK4001

Shipping and Storage

1. All solutions should be clear. If the ambient temperature is low and the solution may form a precipitate, it 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. Wash Solution 2/3 may precipitate crystals after adding ethanol and using for a few days, but it does not affect its use. Simply do not absorb the crystals and absorb the supernatant before use.
4. To avoid the volatilization, oxidation, and pH changes of reagents exposed to air for a long time, the lid of each solution should be promptly closed after use.

Components

Component	Storage	RNK4001 50 Preps
Buffer RLT Plus	RT	50mL
PLANTaid	RT	5mL
Wash Solution 1	RT	12mL Add 28mL anhydrous ethanol before first use
Wash Solution 2/3	RT	10mL Add 42mL anhydrous ethanol before first use
RNase-free H_2O	RT	5mL
Genomic DNA clearance column and collection tube	RT	50
RNase free adsorption column RA and collection tube	RT	50

Note: This reagent kit can be stored at room temperature for 12 months without affecting its effectiveness.

Description

Traditional microRNA extraction kits are made using the principle of guanidine isothiocyanate/phenol/chloroform (TRIzol method) combined with high concentration ethanol silica gel membrane to adsorb small fragments of microRNA. However, for complex plant species such as cotton, grapes, Populus euphratica, holly, roses, dendrobium, ginseng, rice seeds, ginseng, corn germ, and many other samples, the TRIzol principle cannot even extract the required total RNA, let alone microRNA. Therefore, world-renowned companies such as Qiagen and Invitrogen, which use the Trizol method principle for microRNA extraction kits, are helpless when faced with complex plant samples, and there are simply no complex plant microRNA extraction kits in their product lines. Users have no choice but to use traditional methods or TRIzol extraction, and isopropanol/ethanol precipitation method to extract microRNAs. Although some microRNAs can also be extracted, the precipitation method incurs huge losses or co precipitates with impurities, affecting experimental results. When submitting to international journals, they often face doubts and even have their papers revoked. On the basis of the company's global experience in extracting hundreds of complex plants, this kit innovatively uses a technology route that does not require phenol or chloroform to solve this problem. It can extract microRNAs from most complex plants such as cotton, poplar, holly, rose, and Danshen. Of course, this kit is also suitable for various simple samples such as Arabidopsis, rice, wheat, tobacco, and rice.

The unique lysis buffer rapidly lyses cells and inactivates cell RNA enzymes. Plant RNA enhancer PLANTaid helps bind to polysaccharides and polyphenols, which are removed by centrifugation. The lysis mixture is then passed through a genomic DNA

clearance column, where genomic DNA is cleared and RNA (including microRNA) is selectively filtered. After adjusting the binding conditions of the filtered RNA with ethanol, the RNA selectively adsorbs onto the silica membrane inside the centrifuge column in a high salt state. Then, a series of special rinsing centrifugation steps are performed to quickly remove cellular metabolites, proteins, and other impurities. Finally, low salt RNase free H20 elutes pure RNA (including microRNA) from the silica membrane.

The separation and enrichment of microRNA components and total RNA (>200 nt) can also be obtained separately by using the extraction operation steps.

Application

Suitable for rapid extraction of plant microRNA or separate extraction of microRNA/total RNA.

Features

1. Completely avoid using toxic reagents such as phenol and chloroform, and do not require steps such as ethanol precipitation.
2. Fast, simple, and the operation of a single sample can generally be completed within 30 minutes.
3. The unique plant RNA enhancer can effectively bind to polysaccharide polyphenols and improve clearance efficiency.
4. Multiple column rinses ensure high purity, with a typical OD_{260}/OD_{280} ratio of 2.0-2.2, which can be used for RT-PCR, Northern blot, and various experiments.

Note

1. All centrifugation steps can be completed at room temperature (4℃ centrifugation is also possible), using traditional desktop centrifuges with a speed of up to 13000 rpm, such as Eppendorf 5415C or similar centrifuges.
2. Need to bring your own ethanol, β - mercaptoethanol, and 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 result in DNA residue or reduced yield. When starting to explore 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 experimental conditions of the sample.
4. Buffer RLT Plus and Wash Solution 1 contain irritating compounds. Wear latex gloves during operation to avoid contact with skin, eyes, and clothing. If it comes into contact with the skin or eyes, rinse with plenty of water or saline solution.
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). Due to the use of our company's unique buffer system and genomic DNA clearance column technology, the vast majority of DNA has been cleared. In some special cases, trace genomic DNA residues need to be cleared, and the following DNA enzyme digestion methods can be used.
   5.1. The RNA/microRNA extracted by traditional DNA enzyme digestion is directly used for subsequent experiments after heat inactivation of DNA enzyme.
   5.2. The RNA/microRNA extracted by traditional DNA enzyme digestion was cleaned and purified using an RNA cleaning purification kit (but the second step of adding 250μL anhydrous ethanol needs to be changed to adding 700μL anhydrous ethanol), and then used for subsequent experiments.
   5.3. Directly perform DNase I column digestion treatment on the adsorption column RA.
6. When encountering samples with particularly complex secondary metabolites such as polysaccharides, polyphenols, starch, etc., such as grape fruits, rice seeds, and when the RLT Plus lysis solution is not effective, it is recommended to purchase specialized lysis solution CLB. Cracking solution CLB is the strongest formula developed by our company for particularly complex polysaccharide polyphenol plant samples. The vast majority of complex samples that cannot be extracted by cracking solution RLT Plus can be successfully extracted.

Protocol(Please read the precautions before the experiment)

Note:1)Please add the specified amount of anhydrous ethanol to Wash Solution 1 and Wash Solution 2/3 bottles before the first use!

2)For plant tissues that are particularly rich in RNA enzymes or polyphenols: Before operation, add β - mercaptoethanol to buffer RLT Plus to a final concentration of 1%, such as adding 10μL β - mercaptoethanol to 1mL RLT Plus. It is best to use and prepare this cracking solution on site. The prepared RLT Plus can be stored at 4℃ for one month.

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, take 100mg-200mg and quickly cut it into small pieces and place them in a mortar (frozen or liquid nitrogen stored samples can be directly weighed and taken 100mg-200mg and placed in the mortar). Add 10 volumes (1mL) of RLT Plus and 1 volume (100μL) of PLANTaid and grind them thoroughly at room temperature to form a homogenate. Note that the tissue and buffer RLT Plus should be quickly ground to immediately come into full contact and inhibit RNAse activity.

   Note: PLANTaid is an essential component for extracting pigments, a secondary metabolite of polysaccharides and polyphenols, from difficult samples.

   1.2. Transfer the lysate into a centrifuge tube, vigorously shake and oscillate for 15 seconds, centrifuge at 13000rpm for 5-10 minutes, and precipitate fragments that cannot be lysed and PLANTaid bound with polysaccharide polyphenols.
   1.3. Take 480μL of lysate supernatant (more supernatant can be taken within the capacity of the genomic DNA clearance column to increase yield, and if there is a large amount of residual genomic DNA, the amount of supernatant can be appropriately reduced) and add the lysate supernatant to the DNA clearance column (the clearance column is placed in a collection tube).
   1.4. Immediately follow step 3 of the operation procedure.
2. Liquid nitrogen grinding method (recommended for extracting complex and easily degradable samples):
   2.1. Take 500μL of buffer RLT Plus, transfer it into a 1.5mL centrifuge tube, add 50μL 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 50mg-100mg of fine powder and transfer it into the centrifuge tube containing RLT Plus and PLANTaid mentioned above. Immediately shake vigorously by hand for 20 seconds to fully lyse.
   2.3. Using a suction head to blow and mix can help with lysis or vigorously vortex 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 13000 rpm for 5-10 minutes to precipitate fragments that cannot be lysed and PLANTaid bound with polysaccharide polyphenols.
   2.5. Take the lysate supernatant (more supernatant can be taken within the capacity of the genomic DNA clearance column to increase yield) and add the lysate supernatant to the DNA clearance column (which is placed in a collection tube).
   2.6. Immediately follow step 3 of the operation procedure.

   Note: The above liquid nitrogen grinding method allows users to double the processing as needed, which can increase production. That is to say, use 1ml of buffer RLT Plus, 100μL of PLANTaid, and 100mg-200mg samples.
3. Immediately centrifuge at 13000 rpm for 60 seconds and collect the filtrate (RNA in the filtrate).
4. Use a micropipette to accurately estimate the volume of the filtrate (around 480μL, which should be subtracted from the volume lost during filtration). Add 1.25 times the volume of anhydrous ethanol, which may cause precipitation but does not affect the extraction process. Immediately blow and mix well, and do not centrifuge.
5. Immediately add the mixture (less than 700μL each time, which can be added in two separate portions) to an adsorption column RA, centrifuge at 13000 rpm for 30 seconds, and discard the waste liquid. **Ensure that all the liquid after centrifugation is filtered and there is no residue on the membrane. If necessary, increase the centrifugal force and time.**
6. Add 700μL of Wash Solution 1 (please check if anhydrous ethanol has been added first!)Centrifuge at 12000rpm for 30 seconds and discard the waste liquid.
7. Add 500μL Wash Solution 2/3 (please check if anhydrous ethanol has been added first!)Centrifuge at 12000 rpm for 30 seconds and discard the waste liquid. Add 500μL of Wash Solution 2/3 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 the

rinse solution as much as possible to avoid residual ethanol in the rinse solution inhibiting downstream reactions.

9. Remove the adsorption column RA and place it in an RNase free centrifuge tube. Add 30-50μL of RNase free water to the middle part of the adsorption membrane according to the expected RNA yield, let it stand at room temperature for 1 minute, and centrifuge at 12000 rpm for 1 minute. **Adding the eluent back to the adsorption column and repeating the elution step can increase the yield by about 10-15%.**
10. The obtained RNA/microRNA can be immediately used for downstream reactions or stored at low temperature as soon as possible.

Appendix 1: MicroRNA relative enrichment method (microRNA/total RNA with microRNA removed extracted separately)

Note:1)Please add the specified amount of anhydrous ethanol to the buffer RW bottle before the first use!

2)For plant tissues that are particularly rich in RNA enzymes or polyphenols: Before operation, add β - mercaptoethanol to buffer RLT Plus to a final concentration of 1%, such as adding 10μL β - mercaptoethanol to 1mL RLT Plus. It is best to use and prepare this cracking solution on site. The prepared RLT Plus can be stored at 4℃ for one month.

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, take 100mg-200mg and quickly cut it into small pieces and place them in a mortar (frozen or liquid nitrogen stored samples can be directly weighed and taken 100mg-200mg and placed in the mortar). Add 10 volumes (1mL) of RLT Plus and 1 volume (100μL) of PLANTaid and grind them thoroughly at room temperature to form a homogenate. Note that the tissue and buffer RLT should be quickly ground to immediately come into full contact and inhibit RNAse activity.

   Note: PLANTaid is an essential component for extracting pigments, a secondary metabolite of polysaccharides and polyphenols, from difficult samples.

   1.2. Transfer the lysate into a centrifuge tube, vigorously shake and oscillate for 15 seconds, centrifuge at 13000rpm for 5-10 minutes, and precipitate fragments that cannot be lysed and PLANTaid bound with polysaccharide polyphenols.
   1.3. Take 480μL of lysate supernatant (more supernatant can be taken within the capacity of the genomic DNA clearance column to increase yield, and if there is a large amount of residual genomic DNA, the amount of supernatant can be appropriately reduced) 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, do not centrifuge.
   1.4. Immediately proceed to step 3 of the enrichment method.
2. Liquid nitrogen grinding method (recommended for extracting complex and easily degradable samples):
   2.1. Take 500μL of buffer RLT Plus, transfer it into a 1.5mL centrifuge tube, add 50μL 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 50mg-100mg of fine powder and transfer it into the centrifuge tube containing RLT Plus and PLANTaid mentioned above. Immediately shake vigorously by hand for 20 seconds to fully lyse.
   2.3. Using a suction head to blow and mix can help with lysis or vigorously vortex 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 13000 rpm for 5-10 minutes to precipitate fragments that cannot be lysed and PLANTaid bound with polysaccharide polyphenols.
   2.5. Transfer the lysate supernatant (more supernatant can be taken within the capacity of the genomic DNA clearance column to increase yield) 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, do not centrifuge.
   2.6. Immediately proceed to step 3 of the enrichment method.

Note: The above liquid nitrogen grinding method allows users to double the processing as needed, which can increase production. That is to say, use 1ml of buffer RLT Plus, 100μL of PLANTaid, and 100mg-200mg samples.

3. Add the mixture (less than 720μL each time, which can be added in two separate portions) into a genome clearing column (place the clearing column in a collection tube), centrifuge at 13000 rpm for 2 minutes, and retain the filtrate (microRNA in the filtrate). **At this point, the filtrate contains microRNA, and on the genomic DNA clearance column is the total RNA from which microRNA has been removed (excluding microRNA). If necessary, rinse according to the standard operating steps 6-10 to recover the total RNA from which microRNA has been removed.**
4. Use a micropipette to accurately estimate the volume of the filtrate, add an equal volume of anhydrous ethanol (which must be at room temperature), vortex or blow thoroughly, and do not centrifuge.
5. Immediately add the mixture (less than 700μL each time, and can be added in two separate portions) to an adsorption column RA, centrifuge at 13000 rpm for 2 minutes, and discard the waste liquid. **Ensure that all the liquid after centrifugation is filtered and there is no residue on the membrane. If necessary, increase the centrifugal force and time.**
6. Follow the standard operating steps 6-10 to rinse and elute to obtain enriched microRNAs.

Note: Different experiments can choose different methods, such as Northern Blot or expression chip profiling, which can extract total RNA including microRNA. The microRNAs extracted by relative enrichment methods may reduce the amplification background of some downstream experiments due to the removal of larger fragments of mRNA and rRNA. When the background is high or non-specific amplification is abundant, relative enrichment methods can be used to extract microRNAs.

Appendix 2: DNA enzyme column digestion (please refer to the instructions of RNK3401 DNase I column digestion kit for details)

1. Follow the steps listed earlier until Step 5 is completed.
2. Take 45μL of DNase I buffer and 5μL of RNase free DNase I and gently mix them in a centrifuge tube to form the working solution (when processing multiple centrifuge columns, the working solution should be prepared by scaling up according to the proportion).
3. Add 350μL of Wash Solution 1 to the adsorption column RA, centrifuge at 12000 rpm for 30 seconds, discard the waste liquid, and place the adsorption column in the recovery manifold.
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. Be careful to directly immerse the working liquid droplet in the center of the membrane and fully immerse it around the membrane to make contact with the membrane. Do not let the working liquid droplet hang on the O-ring or centrifuge column wall, or hang on the gasket to prevent sufficient contact with the membrane.
5. Add 350μL of Wash Solution 1 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 header.
6. Follow operation step 7 to complete the subsequent steps.

Appendix 3: EASYpin Plant MicroRNA Rapid Extraction Kit Using Buffer CLB Operation Steps

Note:1)Please add the specified amount of anhydrous ethanol to Wash Solution 1 and Wash Solution 2/3 bottles before the first use!

2)Take 1mL of buffer CLB into a centrifuge tube (if there is precipitation or precipitation, it needs to be dissolved again in a 65°C water bath), and add 5% ß - mercaptoethanol (1mL CLB plus 50μL ß - mercaptoethanol) to the buffer CLB. Preheat in a 65°C water bath after reversing and mixing.

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 for samples with less moisture such as seeds and leaves, and more can be added for samples with more moisture such as watermelon) to a preheated buffer CLB (already added with ß - mercaptoethanol) centrifuge tube. Immediately vortex vigorously for 30-60 seconds or use a suction head to blow and mix well for cracking. Place it back in a 65°C water bath for a short period of time (5-10 min, slightly longer for 10 minutes may

increase yield), and occasionally invert 1-2 times in between to help with cracking. **ß - mercaptoethanol is a key component of the cracking solution CLB, and if necessary, the final concentration can be increased to 10-20%.**

3. After shaking and mixing, centrifuge at room temperature of 13000rpm for 10 minutes.
4. Transfer the lysate supernatant (more supernatant can be taken within the capacity of the genomic DNA clearance column to increase yield) to a new centrifuge tube. **If there are floating objects on the surface of the supernatant, use a suction head to pick up and absorb the liquid below.**
5. Immediately proceed with the next steps.