CYRN40-CHANGYU EASYspin Plant microRNA Kit

Catalog number	Package
CYRN4001	50 preps

Storage and shipping: 15-25 °C. Stable for 6 months. Solutions should be clear; if a precipitate forms at low temperature, heat in a 37 °C water bath for a few minutes until clear. Wash Solution 2/3 may form crystals a few days after adding ethanol; avoid the crystals and use the supernatant. Keep all solutions tightly capped after use.

Applications

For rapid extraction of plant microRNA, or for separate extraction of microRNA and total RNA.

Kit components

Component	Storage	50 preps (CYRN4001)
Lysis Buffer RLT Plus	15-25 °C	50 ml
PLANTaid	15-25 °C	5 ml
Wash Solution 1	15-25 °C	12 ml (add 28 ml absolute ethanol before first use)
Wash Solution 2/3	15-25 °C	10 ml (add 42 ml absolute ethanol before first use)
RNase-free H2O	15-25 °C	10 ml
Genomic DNA removal columns and collection tubes	15-25 °C	50 sets
RNase-free spin columns RA and collection tubes	15-25 °C	50 sets

Description

Conventional microRNA kits are based on guanidinium thiocyanate/phenol/chloroform (TRIzol) extraction followed by binding of small RNAs to a silica membrane in high-concentration ethanol. Complex plant species such as cotton, grape, Populus euphratica, holly, rose, Dendrobium, Salvia miltiorrhiza, rice seed, ginseng and maize germ cannot be extracted reliably by this route, and microRNA kits based on TRIzol are therefore not effective for such samples. This kit uses a phenol- and chloroform-free route. A dedicated lysis buffer rapidly lyses cells and inactivates cellular RNases; PLANTaid binds polysaccharides and polyphenols, which are removed by centrifugation; the lysate is passed through a genomic DNA removal column that retains genomic DNA while RNA, including microRNA, flows through. The filtered RNA is adjusted with ethanol and binds selectively to the silica membrane under high-ionic-strength conditions; special wash buffers remove metabolites, proteins and other impurities, and purified RNA, including microRNA, is eluted in low-salt RNase-free H2O. A separate procedure can enrich the microRNA fraction and recover total RNA (larger than 200 nt) separately.

Features

No toxic phenol or chloroform and no ethanol precipitation step are required.

Fast and simple: a single sample is usually processed within 30 minutes.

PLANTaid effectively binds polysaccharides and polyphenols and improves their removal.

Repeated column washes ensure high purity; typical A260/A280 is 2.0-2.2, suitable for RT-PCR, Northern blot and other applications.

Notes before starting

Note: All centrifugation steps can be carried out at room temperature (4 °C is also acceptable) at 13,000 rpm in a conventional benchtop centrifuge such as an Eppendorf 5415C.

Note: Ethanol, β-mercaptoethanol and an optional mortar must be supplied by the user.

Note: Do not exceed the capacity of genomic DNA removal column DA or RNA column RA; otherwise DNA residue or reduced yield will result. When establishing conditions for an unknown sample, start with less material and adjust afterwards.

Note: Lysis Buffer RLT Plus and Wash Solution 1 contain irritants. Wear gloves and avoid contact with skin, eyes and clothing. Rinse affected areas with plenty of water or saline.

Note: Trace genomic DNA cannot be excluded completely by any total-RNA procedure, and even DNase digestion is not 100% effective. This kit uses a dedicated buffer system and genomic DNA removal column that remove most DNA. If trace genomic DNA must be removed: (1) digest with conventional DNase, heat-inactivate and use directly; (2) digest with conventional DNase and purify with the RNAclean RNA Clean Kit (CYRN14; in step 2 change 250 µl of absolute ethanol to 700 µl); (3) perform on-column DNase I digestion on column RA using the CYRN34 DNase I on-column digestion kit (in that protocol replace Deproteinization Buffer RW1 with Wash Solution 1).

Note: For very complex samples rich in polysaccharides, polyphenols, starch or secondary metabolites (for example grape fruit or rice seed) where Lysis Buffer RLT Plus performs poorly, use the dedicated CLB lysis buffer (see Appendix 3); CLB is the strongest formulation for very complex polysaccharide/polyphenol plant samples.

❖ Procedure

Read the notes before starting. Before first use, add the indicated amount of absolute ethanol to Wash Solution 1 and Wash Solution 2/3. For plant tissues very rich in RNase or polyphenols, add β-mercaptoethanol to Lysis Buffer RLT Plus to a final concentration of 1% (10 µl per 1 ml RLT Plus) before use; prepare fresh, and the prepared buffer can be stored at 4 °C for one month.

1. Direct grinding (recommended for simple plant samples; liquid nitrogen grinding can also be used for simple samples)

a. Weigh 100 mg of fresh plant tissue and cut it into small pieces into a mortar (frozen or liquid-nitrogen-stored samples can be weighed directly). Add 10 volumes (1 ml) of RLT Plus and 1 volume (100 µl) of PLANTaid and grind thoroughly at room temperature. Grind quickly so that the tissue contacts the lysis buffer immediately and RNase activity is inhibited. PLANTaid is essential for difficult samples rich in polysaccharides, polyphenols, secondary metabolites and pigments.

b. Transfer the lysate to a centrifuge tube, shake vigorously for 15 seconds and centrifuge at 13,000 rpm for 5-10 minutes to pellet unlysed debris and PLANTaid-bound impurities.

c. Take 480 µl of the supernatant (more may be taken if the capacity of the genomic DNA removal column is not exceeded, which increases yield; reduce the volume if genomic DNA residue is high) and load it onto the genomic DNA removal column placed in a collection tube.

d. Proceed immediately to step 3.

2. Liquid nitrogen grinding (recommended for complex or easily degraded samples)

a. Place 500 µl of RLT Plus in a 1.5 ml centrifuge tube, add 50 µl of PLANTaid and mix.

b. Grind plant tissue in liquid nitrogen to a fine powder, transfer 50 mg of powder to the tube containing RLT Plus and PLANTaid and shake vigorously by hand for 20 seconds.

c. Pipette or vortex vigorously until a satisfactory homogenate is obtained (or homogenize with an electric homogenizer for 30 seconds) to shear DNA, reduce viscosity and increase yield.

d. Centrifuge at 13,000 rpm for 5-10 minutes to pellet unlysed debris and PLANTaid-bound impurities.

e. Take the supernatant (more may be taken if column capacity is not exceeded) and load it onto the genomic DNA removal column placed in a collection tube.

f. Proceed immediately to step 3.

The liquid nitrogen protocol can be scaled up (1 ml RLT Plus, 100 µl PLANTaid and 100 mg sample) to increase yield.

3. Centrifuge immediately at 13,000 rpm for 60 seconds and collect the filtrate, which contains the RNA.

4. Estimate the filtrate volume accurately with a micropipette (about 480 µl, minus volume lost during filtration) and add 1.25 volumes of absolute ethanol. A precipitate may form but does not affect the procedure; mix immediately by pipetting and do not centrifuge.

5. Immediately load the mixture (no more than 700 µl per load; larger volumes can be loaded in two portions) onto spin column RA placed in a collection tube and centrifuge at 13,000 rpm for 30 seconds. Discard the flow-through. Make sure all liquid has passed through the membrane; if necessary, increase the centrifugal force and time.

6. Add 700 µl of Wash Solution 1 (check that ethanol has been added), centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

7. Add 500 µl of Wash Solution 2/3 (check that ethanol has been added), centrifuge at 12,000 rpm for 30 seconds and discard the flow-through. Repeat once with another 500 µl of Wash Solution 2/3.

8. Return column RA to the empty collection tube and centrifuge at 13,000 rpm for 2 minutes to remove residual wash buffer, since residual ethanol can inhibit downstream reactions.

9. Remove column RA and place it in an RNase-free centrifuge tube. Apply 30-50 µl of RNase-free water to the centre of the membrane, leave at room temperature for 1 minute and centrifuge at 12,000 rpm for 1 minute. Reloading the eluate onto the column for a second elution increases yield by about 10-15%.

10. The RNA/microRNA can be used immediately in downstream reactions or stored at low temperature as soon as possible.

❖ Appendix 1: microRNA enrichment (separate extraction of microRNA and microRNA-depleted total RNA)

Add ethanol to Wash Solution 1 and Wash Solution 2/3 before first use. For RNase- or polyphenol-rich tissues, add β-mercaptoethanol to RLT Plus to 1% before use as described above.

1. Prepare the lysate by direct grinding or liquid nitrogen grinding as in the standard procedure, steps 1-2, but take the supernatant into a new tube and add 0.5 volume of absolute ethanol instead of loading it onto the genomic DNA removal column. A precipitate may form; mix immediately and do not centrifuge.

2. Proceed immediately to step 3 of the enrichment procedure.

3. Load the mixture (no more than 720 µl per load) onto a genomic DNA removal column placed in a collection tube and centrifuge at 13,000 rpm for 2 minutes. Keep the filtrate, which contains microRNA. The column retains microRNA-depleted total RNA; if desired, wash and elute it according to standard steps 6-10 to recover microRNA-depleted total RNA.

4. Estimate the filtrate volume accurately and add 0.65 volume of absolute ethanol (at room temperature). Vortex or pipette thoroughly to mix; do not centrifuge.

5. Immediately load the mixture (no more than 700 µl per load) onto spin column RA placed in a collection tube and centrifuge at 13,000 rpm for 2 minutes. Discard the flow-through.

6. Wash and elute according to standard steps 6-10 to obtain enriched microRNA.

Note: Different experiments may require different approaches. For example, Northern blot or expression array analysis may use total RNA that includes microRNA. Enriched microRNA lacks large mRNA and

rRNA species and may reduce amplification background in some downstream assays; when background or non-specific amplification is high, enriched microRNA can be tried.

❖ Appendix 2: On-column DNase digestion (see the CYRN34 DNase I on-column digestion kit for details)

1. Follow the standard procedure up to and including step 5.

2. Mix 45 μl of DNase I buffer and 5 μl of RNase-free DNase I gently to prepare the working solution (scale up proportionally for multiple columns).

3. Add 350 μl of Wash Solution 1 to column RA, centrifuge at 12,000 rpm for 30 seconds, discard the flow-through and return the column to the collection tube.

4. Apply 50 μl of DNase I working solution to the centre of the membrane and leave at room temperature (20-30 °C) for 15 minutes. Apply the solution directly to the membrane; do not let it run onto the O-ring or column wall.

5. Add 350 μl of Wash Solution 1, centrifuge at 12,000 rpm for 30-60 seconds, discard the flow-through and return the column to the collection tube.

6. Continue with step 7 of the standard procedure.

❖ Appendix 3: Protocol using CLB lysis buffer

Add ethanol to Wash Solution 1 and Wash Solution 2/3 before first use. Transfer 1 ml of CLB lysis buffer to a centrifuge tube (if CLB has precipitated at low temperature, redissolve in a 65 °C water bath) and add β-mercaptoethanol to 5% (50 μl per 1 ml CLB). Mix by inversion and prewarm in a 65 °C water bath.

1. Grind fresh or -70 °C-frozen material in liquid nitrogen to a fine powder.

2. Transfer 100-150 mg of powder (100 mg for low-moisture samples such as seeds and leaves; more for high-moisture samples such as watermelon) to the prewarmed CLB tube and vortex vigorously for 30-60 seconds or mix by pipetting. Return briefly to the 65 °C water bath (5-10 minutes; 10 minutes may improve yield), inverting once or twice. β-Mercaptoethanol is a key component of CLB; when necessary its final concentration can be increased to 10-20%.

3. Vortex to mix, then centrifuge at room temperature at 13,000 rpm for 10 minutes.

4. Take the supernatant (more may be taken if column capacity is not exceeded) into a new tube. If material floats on the surface, pipette through it and take the liquid below.

5. Continue with the subsequent procedure steps (load the supernatant onto the genomic DNA removal column and proceed as in the standard procedure).

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