CYRN42-CHANGYU EASYspin Plus Large-Scale Plant RNA Kit

Catalog number	Package
CYRN4201	10 preps

Storage and shipping: 15-25 °C. Stable for 6 months. PLANTaid is shipped at room temperature and should be stored at 4 °C. Solutions should be clear; if a precipitate forms at low temperature, heat in a 37 °C water bath for a few minutes until clear. Do not store at 4 °C or -20 °C. Keep all solutions tightly capped after use.

Applications

For rapid extraction of total RNA from plant tissues and cells. The dedicated genomic DNA removal column effectively removes residual gDNA, so generally no DNase digestion is needed and the RNA can be used directly in PCR and real-time PCR.

Kit components

Component	Storage	10 preps (CYRN4201)
Lysis Buffer RLT	15-25 °C	100 ml
Lysis Buffer RLT Plus	15-25 °C	50 ml
Deproteinization Buffer RW1	15-25 °C	120 ml
Wash Buffer RW	15-25 °C	25 ml × 2 (add ethanol before first use)
RNase-free H2O	15-25 °C	10 ml
PLANTaid	Ship at 15-25 °C; store at 4 °C	10 ml × 2
Genomic DNA removal columns and collection tubes	15-25 °C	10 sets
RNase-free spin columns RA and collection tubes	15-25 °C	10 sets

Description

Based on the EASYspin phenol- and chloroform-free RNA extraction technology, this kit adds a genomic DNA removal column that effectively removes residual gDNA, so the RNA generally requires no DNase digestion and can be used directly in PCR and real-time PCR. A dedicated lysis buffer with β-mercaptoethanol rapidly lyses cells and inactivates cellular RNases. PLANTaid binds polysaccharides and polyphenols, which are removed by centrifugation. The lysate is adjusted with ethanol and applied to the genomic DNA removal column, where genomic DNA is retained and RNA is selectively eluted through. The filtered RNA is adjusted with ethanol again and binds selectively to the silica membrane under high-ionic-strength conditions; rapid wash and centrifugation steps remove metabolites, proteins and other impurities, and purified RNA is eluted in low-salt RNase-free H2O.

Features

The silica membrane is selected from a world-leading supplier; variation in binding capacity between columns is minimal and reproducibility is high.

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

Fast and simple: a single sample is usually processed within 60 minutes.

The dedicated plant RNA extraction aid effectively binds polysaccharides and polyphenols and improves their removal.

Repeated column washes ensure high purity; typical A260/A280 is 1.9-2.0 with little residual DNA, suitable for RT-PCR, Northern blot and other applications.

Notes before starting

Note: All centrifugation steps can be carried out at room temperature in a centrifuge that accepts 50 ml tubes.

Note: Ethanol, an optional disposable syringe and a mortar must be supplied by the user.

Note: Lysis Buffer RLT, Lysis Buffer RLT Plus and Deproteinization Buffer RW1 contain irritants. Wear gloves and avoid contact with skin, eyes and clothing. Rinse affected areas with plenty of water or saline.

Note: Complete lysis directly affects RNA quality and yield. Lysis Buffer RLT, whose main component is guanidinium thiocyanate, suits most plant tissues. Some tissues (for example the milky endosperm of maize) or filamentous fungi contain secondary metabolites that cause the sample to solidify in guanidinium thiocyanate and prevent RNA extraction; an alternative lysis buffer RLC is available on request.

Note: Trace genomic DNA cannot be excluded completely by any total-RNA procedure. The EASYspin buffer system and membrane remove most DNA, and trace DNA has little effect in most RT-PCR amplifications. For stringent mRNA expression analysis such as real-time PCR: use intron-spanning primers; use primer pairs that give different product sizes on genomic DNA and cDNA; treat the RNA with RNase-free DNase I; the kit can also clean up RNA after DNase I treatment; or perform DNase I treatment on column RA before the RW1 wash.

Note: RNA integrity: run on a 1.2% agarose gel in 0.5× TBE at 150 V for 15 minutes. Because 70-80% of cellular RNA is rRNA, prominent rRNA bands should be visible under UV. Animal rRNA is about 5 kb and 2 kb (28S and 18S); the largest rRNA band should be 1.5-2.0 times as bright as the next largest, otherwise the RNA is degraded. Purity: high-quality RNA gives A260/A280 of 1.8-2.1 in 10 mM Tris, pH 7.5; readings in water may be 1.5-1.9. Concentration: (ng/µl) = A260 × dilution factor n × 40.

❖ Procedure

Before first use, add the indicated amount of absolute ethanol to Wash Buffer RW.

1. Direct grinding (recommended)

a. Weigh 1-2 g 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 (10 ml) of RLT and 1 volume (1 ml) 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 and polyphenols; for ordinary plant tissues it may be omitted, which may increase RNA yield.

b. Transfer the lysate to a centrifuge tube, shake vigorously for 15 seconds and centrifuge at 10,000-13,000 × g for 10 minutes (extend the time if the centrifuge speed is lower) to pellet unlysed debris and PLANTaid-bound impurities. Carefully transfer the supernatant to a new tube and note its volume.

c. Add half a volume of absolute ethanol (0.5 volume). A precipitate may form; it does not affect the procedure. Vortex immediately to mix and do not centrifuge.

d. Proceed immediately to step 3.

2. Liquid nitrogen grinding

a. Place 10 ml of RLT in a 50 ml centrifuge tube, add 1 ml of PLANTaid and mix.

b. Grind plant tissue in liquid nitrogen to a fine powder, transfer 1-2 g of powder to the tube containing RLT and PLANTaid and shake vigorously by hand for 20 seconds. Incubation at 56 °C for 1-3 minutes aids lysis, but starch-rich plants must not be incubated because the elevated temperature can cause starch to swell.

c. Homogenize the lysate by drawing it up and down 10 times through a disposable 5 ml syringe with a blunt 0.9 mm needle, or until a satisfactory homogenate is obtained (or homogenize with an electric homogenizer for 30 seconds).

d. Centrifuge at 10,000-13,000 × g for 10 minutes to pellet unlysed debris and PLANTaid-bound impurities. Transfer all of the supernatant to a new tube.

e. Estimate the supernatant volume accurately, add 0.5 volume of absolute ethanol (a precipitate may form; it does not affect the procedure), vortex immediately to mix and do not centrifuge.

f. Proceed immediately to step 3.

3. Load the mixture onto a genomic DNA removal column placed in a collection tube and centrifuge at 10,000-13,000 × g for 5 minutes (make sure all liquid has passed through and no liquid remains on the membrane; otherwise increase speed and time). Discard the flow-through.

4. Place the genomic DNA removal column in a clean 50 ml centrifuge tube (an ordinary clean tube is sufficient; a clean collection tube from a new RNA column set can also be used). Add 5 ml of Lysis Buffer RLT Plus to the column and centrifuge at 13,000 rpm for 2 minutes. Collect the filtrate, which contains the RNA. Estimate the filtrate volume accurately (usually about 4-5 ml, minus volume lost during filtration) and add 0.5 volume of absolute ethanol. A precipitate may form; mix immediately by pipetting and do not centrifuge.

5. Load the mixture onto RNA spin column RA placed in a collection tube and centrifuge at 10,000-13,000 × g for 5 minutes (make sure all liquid has passed through and nothing remains on the membrane; otherwise increase speed and time). Discard the flow-through.

6. Add 6 ml of Deproteinization Buffer RW1, leave at room temperature for 1 minute, centrifuge at 12,000 × g for 3 minutes and discard the flow-through.

7. Add 10 ml of Wash Buffer RW (check that ethanol has been added), centrifuge at 10,000-13,000 × g for 1-2 minutes and discard the flow-through. Repeat once with another 10 ml of Wash Buffer RW.

8. Return column RA to the empty collection tube and centrifuge at 13,000 × g for 5 minutes to dry the membrane. Use a pipette tip to remove any residual ethanol between the inner ring and the column wall, then air-dry at room temperature or in an oven for a few minutes.

9. Remove column RA and place it in an RNase-free centrifuge tube. Apply 500 µl-1 ml of RNase-free H2O to the centre of the membrane (prewarming to 70-90 °C improves elution), leave at room temperature for 3 minutes and centrifuge at 12,000 × g for 2 minutes.

10. If the expected RNA yield is greater than 0.6 mg, add 300-500 µl of RNase-free H2O and repeat step 9, then combine the two eluates; alternatively, reload the first eluate onto the column if a higher concentration is needed. Two separate elutions give 15-30% more total RNA but a lower concentration than a single elution with the combined first eluate; choose according to your needs.

This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.