CYRN38-CHANGYU EASYspin Plus Plant RNA Kit (Polysaccharide/Polyphenol)

Catalog number	Package
CYRN3801	50 preps

Storage and shipping: 15-25 °C. Stable for 12 months at room temperature. Keep all solutions tightly capped after use.

Applications

For rapid extraction of RNA from ordinary plants, polysaccharide- and polyphenol-containing plants, and fungi.

Kit components

Component	Storage	50 preps (CYRN3801)
Lysis Buffer RLT	15-25 °C	50 ml
Lysis Buffer CLB (included)	15-25 °C	8 ml
Lysis Buffer RLT Plus	15-25 °C	25 ml
Deproteinization Buffer RW1	15-25 °C	35 ml
Wash Buffer RW	15-25 °C	10 ml (add ethanol before first use)
RNase-free H2O	15-25 °C	5 ml
PLANTaid	15-25 °C	5 ml
DNA-clear/RNA-bind universal columns and collection tubes	15-25 °C	100 sets

Product update: the genomic DNA removal column and the RNA spin column are the same spin column. Earlier manuals named them according to their role in the procedure; the current manual calls them DNA-clear/RNA-bind universal columns, and the two packages contain the same item.

Description

Building on the EASYspin phenol- and chloroform-free RNA extraction technology, this kit adds a genomic DNA removal column/RNA binding universal column and a dedicated buffer formulation that effectively remove residual gDNA, so the RNA generally requires no DNase digestion and can be used in reverse transcription PCR and real-time PCR. A dedicated lysis buffer 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 universal column, where RNA is selectively eluted through while residual DNA remains bound and is discarded with the column. 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

No toxic phenol, chloroform or β-mercaptoethanol is used.

Simple and rapid: a single sample is usually processed within 20 minutes.

PLANTaid effectively binds polysaccharides and polyphenols and improves their removal.

The genomic DNA removal column and dedicated formulation remove residual gDNA effectively without the need for DNase digestion.

Broad adaptability: the procedure has been used with cotton, rose, peony, Arabidopsis, poplar, rice, wheat, soybean, tobacco, maize, holly and hundreds of other plant species, including samples that failed with other kits.

Repeated column washes ensure high purity; typical A260/A280 is 2.1-2.2 with little residual DNA, suitable for RT-PCR, Northern blot and other applications.

Notes before starting

Note: All centrifugation steps are carried out at room temperature at 13,000 rpm.

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: Trace genomic DNA cannot be excluded completely by any total-RNA procedure, and even DNase digestion is not 100% effective. The EASYspin Plus buffer system and genomic DNA removal column remove most DNA, so the RNA generally needs no DNase digestion and can be used directly in reverse transcription PCR and real-time PCR. For samples with very high DNA content or for stringent mRNA expression analysis by real-time PCR: (1) use intron-spanning primers; (2) use primer pairs that give different product sizes on genomic DNA and cDNA; (3) treat the RNA with RNase-free DNase I (CYRN45); the kit can also be used to clean up RNA after DNase I treatment; (4) perform on-column DNase I digestion on the universal column before the RW1 wash (CYRN34).

Note: If the CLB lysis buffer performs well, it can be ordered separately; alternatively, use the CYRN53 EASYspin Plus Polysaccharide/Polyphenol Complex Plant RNA Kit directly.

❖ Procedure

Read the notes before starting. Before first use, add absolute ethanol to Wash Buffer RW as indicated on the label.

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

a. Weigh 100-200 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 Lysis Buffer RLT 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 500 µl of the supernatant (more may be taken if the capacity of the genomic DNA removal column is not exceeded) into a new tube. Add half a volume of absolute ethanol (0.5 volume); a precipitate may form but does not affect the procedure. Mix immediately by pipetting and do not centrifuge.

d. Proceed immediately to step 3 of the procedure.

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

a. Place 500 µl of Lysis Buffer RLT 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 50-100 mg of powder to the tube containing RLT and PLANTaid and shake vigorously by hand for 20 seconds.

c. Pipette up and down 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 the lysate 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) into a new tube. Add half a volume of absolute ethanol (0.5 volume); a precipitate may form but does not affect the procedure. Mix immediately by pipetting and do not centrifuge.

f. Proceed immediately to step 3 of the procedure.

The liquid nitrogen protocol can be scaled up (1 ml RLT, 100 µl PLANTaid and 100-200 mg sample) to increase yield.

3. Load the mixture (no more than 750 µl per load; larger volumes can be loaded in two portions) onto a DNA-clear/RNA-bind universal column placed in a collection tube. Leave for 1 minute, centrifuge at 13,000 rpm for 2 minutes and discard the flow-through.

Make sure all liquid has passed through and nothing remains on the membrane; if necessary, increase the centrifugal force and time.

4. Place the universal column in a clean 2 ml centrifuge tube (an ordinary clean tube is sufficient; a clean collection tube from a new universal column set can also be used). Add 500 µl of Lysis Buffer RLT Plus to the column, centrifuge at 13,000 rpm for 30 seconds, discard the column and keep the filtrate, which contains the RNA.

5. Add 0.5 volume of absolute ethanol (about 250 µl) to the filtrate and mix by pipetting.

Cloudiness or flocculation after adding ethanol is normal; mix immediately and do not centrifuge.

6. Immediately load the mixture onto a new DNA-clear/RNA-bind universal column placed in a collection tube. Leave for 1 minute, centrifuge at 13,000 rpm for 30 seconds and discard the flow-through (the RNA is now bound to the column). Return the column to the collection tube.

7. Add 700 µl of Deproteinization Buffer RW1, leave at room temperature for 1 minute, centrifuge at 13,000 rpm for 30 seconds and discard the flow-through.

8. Add 500 µl of Wash Buffer RW (check that ethanol has been added), centrifuge at 13,000 rpm for 30 seconds and discard the flow-through. Repeat once with another 500 µl of Wash Buffer RW.

9. Return the universal column 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.

10. Transfer the universal column to a new 1.5 ml centrifuge tube. Apply 30-50 µl of RNase-free H2O to the centre of the membrane, leave for 1 minute and centrifuge at 13,000 rpm for 1 minute.

Use an elution volume of at least 30 µl; smaller volumes reduce recovery. To increase RNA concentration, prewarm the RNase-free H2O to 70-90 °C before elution, leave it on the membrane for 1-2 minutes, or reload the first eluate onto the column for a second elution.

❖ Supplementary note 1

Plant species and tissues vary widely in their response to this kit. For some samples the genomic DNA removal column may substantially reduce yield. In that case, follow the procedure of the CYRN09 EASYspin Plant RNA Kit and omit the genomic DNA removal column step; compared with CYRN38, CYRN09 has one fewer column step and may give a higher yield for some samples. If DNA residue increases when using the CYRN09 procedure, add an on-column DNase digestion step (CYRN34) or conventional DNase digestion according to experimental needs.

CYRN09 and CYRN38 use otherwise identical procedures; CYRN09 simply omits steps 3 and 4 and proceeds from step 1 or 2 directly to step 6.

❖ Supplementary note 2

For very difficult plant samples that fail or give low yield with this kit (for example aged rice seed, grape fruit, blue honeysuckle fruit, Dendrobium, lily bulb and potato tuber), Lysis Buffer RLT is not sufficient; use the CYRN53 EASYspin Plus Polysaccharide/Polyphenol Complex Plant RNA Kit. The kit includes 8 ml of the CLB lysis buffer from CYRN53 so that the performance of CLB on your sample can be tested

according to the CYRN53 manual.

❖ Appendix: Protocol using CLB lysis buffer (from CYRN53)

Before first use, add absolute ethanol to Wash Buffer RW. If CLB lysis buffer has precipitated at low temperature, redissolve it in a 65 °C water bath before use.

1. Transfer 1 ml of Lysis Buffer CLB to a centrifuge tube and add β-mercaptoethanol to 5% (50 µl per 1 ml CLB). Mix by inversion and prewarm in a 65 °C water bath.

2. Grind fresh or -70 °C-frozen material in liquid nitrogen to a fine powder. Transfer 100-200 mg of powder (100-150 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 genomic DNA removal column capacity is not exceeded) into a new tube and add half a volume of absolute ethanol (0.5 volume). A precipitate may form but does not affect the procedure. Mix immediately by pipetting and do not centrifuge. If material floats on the surface of the supernatant, pipette through it and take the liquid below.

5. Proceed immediately to step 3 of the main procedure.

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