CYRN09-CHANGYU EASYspin Plant RNA Kit

Catalog number	Package
CYRN0901	50 preps

Storage and shipping: 15-25 °C. Stable for 12 months at room temperature. All solutions should be kept tightly capped after use to avoid evaporation, oxidation and pH drift.

Description

This kit is designed for rapid isolation of total RNA from ordinary plant tissues, polysaccharide- and polyphenol-rich plant tissues, and fungi. A dedicated lysis buffer rapidly lyses cells and inactivates cellular RNases. The plant RNA extraction aid PLANTaid binds polysaccharides and polyphenols, which are removed by centrifugation. Binding conditions are then adjusted with ethanol and RNA selectively binds to the silica membrane of the spin column under high-ionic-strength conditions. After a series of rapid wash and centrifugation steps that remove metabolites, proteins and other impurities, purified RNA is eluted in low-salt RNase-free H2O.

Kit components

Component	Storage	50 preps (CYRN0901)
Lysis Buffer RLT	15-25 °C	50 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
RNase-free spin columns RA and collection tubes	15-25 °C	50 sets

Before first use, add the volume of absolute ethanol indicated on the Wash Buffer RW label and tick the box on the label.

Features

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

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

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

Broad adaptability: the procedure has been used with hundreds of plant species, including cotton, rose, Arabidopsis, poplar and holly, as well as samples that failed with other kits.

Repeated column washes give typical A260/A280 ratios of 2.1-2.2 with little residual DNA; the RNA is 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 in a standard benchtop centrifuge.

Note: Lysis Buffer RLT 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. 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, (1) use intron-spanning primers so that DNA cannot serve as a template; (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 column RA before the RW1 wash, using the on-column DNase I digestion kit (CYRN34).

Note: For complex plant samples that retain more DNA, either perform on-column DNase I digestion (CYRN34) or use the CYRN38 EASYspin Plus Plant RNA Kit, which adds a genomic DNA removal column and in most cases reduces DNA residue below the limit of detection on UV-illuminated gels.

Note: For very difficult plant samples that fail or give low yield with this kit (for example rice seed, grape fruit, blue honeysuckle fruit, lily bulb and potato tuber), Lysis Buffer RLT is not sufficient; use the CYRN53 EASYspin Plus Polysaccharide/Polyphenol Plant RNA Kit, which offers a stronger CLB lysis option for the most complex samples and can markedly improve yield.

❖ 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)

a. Weigh 100-200 mg 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 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 helps remove polysaccharide and polyphenol impurities; for ordinary plants such as rice leaves it may be omitted. In individual cases of low RNA yield or concentration, omitting PLANTaid may improve yield and success rate.

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 polysaccharide/polyphenol-bound PLANTaid.

c. Transfer 500 µl of the supernatant to a new tube (more or all of the supernatant may be taken if the capacity of column RA is not exceeded, which increases yield). Add half a volume of absolute ethanol (0.5 volume). A precipitate may form; it 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 difficult or easily degraded samples)

a. Place 500 µl of 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 to lyse the sample.

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. Transfer the supernatant to a new tube and add half a volume of absolute ethanol (0.5 volume). A precipitate may form; it 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 if needed to increase yield: use 1 ml Buffer RLT, 100 µl PLANTaid and 100-200 mg sample.

3. Load the mixture (no more than 750 μl per load; larger volumes can be loaded in two portions) onto spin column RA placed in a collection tube. Centrifuge at 13,000 rpm for 1 minute and discard the flow-through.

Make sure that the liquid has passed completely through the membrane. If necessary, increase the centrifugal force and time.

4. 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.

5. 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.

6. 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.

7. Transfer column RA 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 nucleic acid recovery. To increase RNA concentration, prewarm the RNase-free H2O to 70-90 °C before elution, leave the water on the membrane for 1-2 minutes at room temperature, or reload the first eluate onto column RA for a second elution.

❖ Appendix 1: On-column DNase I digestion (see the CYRN34 DNase I on-column digestion kit for details)

1. Follow the CYRN09 procedure up to and including step 3.

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 when processing several columns).

3. Add 350 μl of Deproteinization Buffer RW1 to column RA, centrifuge at 13,000 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 so that it spreads evenly; do not let it run onto the O-ring or the column wall.

5. Add 350 μl of Deproteinization Buffer RW1, centrifuge at 13,000 rpm for 30 seconds, discard the flow-through and return the column to the collection tube.

6. Continue with step 5 of the main procedure.

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