# CYRN52-CHANGYU EASYspin Universal Plant RNA Kit (with DNase I)

| Catalog number | Package |
|---|---|
| CYRN5201 | 50 preps |

Storage and shipping: 15-25 °C, except DNase Buffer and RNase-free DNase I at -20 °C. Stable for 12 months when stored as indicated. Do not store room-temperature components at 4 °C or -20 °C, as precipitation affects performance. Keep all solutions tightly capped after use.

## Kit components

| Component | Storage | 50 preps (CYRN5201) |
|---|---|---|
| Lysis Buffer RPA | 15-25 °C | 50 ml |
| Deproteinization Buffer RW1 | 15-25 °C | 40 ml |
| Wash Buffer RW | 15-25 °C | 10 ml (add ethanol before first use) |
| RNase-free H2O | 15-25 °C | 10 ml |
| DNase Buffer | -20 °C | 1.25 ml × 2 |
| RNase-free DNase I | -20 °C | 0.25 ml |
| RNase-free spin columns RA and collection tubes | 15-25 °C | 50 sets |

## Description

A dedicated lysis buffer rapidly lyses cells and inactivates cellular RNases. Binding conditions are adjusted with ethanol and RNA binds selectively to the silica membrane under high-ionic-strength conditions. DNase digests residual DNA directly on the column, and rapid wash and centrifugation steps remove metabolites, proteins and other impurities; purified RNA is eluted in low-salt RNase-free H2O.

## Features

Completely free of toxic β-mercaptoethanol, phenol and chloroform, and no ethanol precipitation step is required.

Simple and rapid: a single sample is usually processed within 40 minutes.

On-column DNase I digestion gives RNA free of DNase, suitable for real-time RT-PCR, next-generation sequencing, arrays, RACE and other applications.

Broad adaptability: suitable for rice, maize, wheat, Arabidopsis, tomato, tobacco and plants containing polysaccharides and polyphenols such as cotton and holly.

Repeated column washes ensure high purity; typical A260/A280 is 2.0-2.2 with no residual DNA, suitable for real-time PCR, RT-PCR, arrays, next-generation sequencing and Northern blot.

## Notes before starting

**Note:** All centrifugation steps are carried out at room temperature at 13,000 rpm in a conventional benchtop centrifuge.

**Note:** Ethanol and an optional mortar must be supplied by the user.

**Note:** Lysis Buffer RPA 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.

## ❖ Procedure

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

1. Direct grinding (recommended)

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 1 ml of Lysis Buffer RPA and grind thoroughly at room temperature. Grind quickly so that the tissue contacts the lysis buffer immediately and RNase activity is inhibited.

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.

c. Take 480 µl of the supernatant (more or all of the supernatant may be taken if the capacity of column RA is not exceeded, which increases yield) 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.

2. Liquid nitrogen grinding

a. Place 500 µl of Lysis Buffer RPA in a 1.5 ml centrifuge tube.

b. Grind plant tissue in liquid nitrogen to a fine powder, transfer 50-100 mg of powder to the RPA tube 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.

e. Take the supernatant (more may be taken if 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.

f. Proceed immediately to step 3. The liquid nitrogen protocol can be scaled up (1 ml RPA and 100-200 mg sample) to increase yield.

3. Load the mixture (no more than 720 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 2 minutes. Discard the flow-through. Make sure all liquid has passed through the membrane; if necessary, increase the centrifugal force and time.

4. Add 350 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. Prepare the DNase I working solution: mix 45 µl of DNase Buffer and 5 µl of RNase-free DNase I gently (scale up proportionally for multiple columns).

6. 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.

7. Add 350 µl of Deproteinization Buffer RW1, centrifuge at 12,000 rpm for 30 seconds, discard the flow-through and return the column to the collection tube.

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 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.

10. 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 (prewarming to 70-90 °C improves yield), leave at room temperature for 1 minute and centrifuge at 12,000 rpm for 1 minute.

11. If the expected RNA yield is greater than 30 μg, add 30-50 μl of RNase-free water and repeat step 10, 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.

If the RNA is only used for routine reverse transcription or gene cloning and not for real-time PCR, the on-column DNase digestion can be omitted: in step 4 use 700 μl of Deproteinization Buffer RW1 instead of 350 μl, and skip steps 5-7.

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