CYRN69-CHANGYU FASTeasy Universal Plant & Fungi RNA Kit

Catalog number	Package
CYRN6901	50 preps

Storage and shipping: 15-25 °C. Stable for 12 months. Do not store at 4 °C or -20 °C, as precipitation affects performance. 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 (CYRN6901)
Lysis Buffer FEA	15-25 °C	30 ml
PlantAid	15-25 °C	3 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	5 ml
DNA-clear/RNA-bind universal columns and collection tubes	15-25 °C	100 sets

Description

This kit rapidly extracts RNA from ordinary plants, polysaccharide/polyphenol-containing plants and fungi. The dedicated genomic DNA removal/RNA binding universal column and a special formulation generally eliminate the need for DNase digestion and effectively remove residual gDNA. The resulting RNA has no significant DNA residue and can be used directly in reverse transcription and real-time PCR or in high-throughput sequencing library preparation.

Features

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

Simple and rapid: a single sample is usually processed within 12 minutes.

The genomic DNA removal/RNA binding universal column effectively removes residual gDNA, giving highly pure RNA with typical A260/A280 of 2.1-2.2; generally no DNase digestion is needed, and the RNA is suitable for reverse transcription PCR, real-time PCR and high-throughput sequencing library preparation.

Broad adaptability: the procedure has been used with cotton, rose, Arabidopsis, rice, tobacco, poplar and hundreds of other samples, including some that failed with other kits. For very complex polysaccharide/polyphenol samples that perform poorly, use the CYRN53 EASYspin Plus Complex Polysaccharide/Polyphenol Plant RNA Kit.

Notes before starting

Note: All centrifugation steps can be 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 FEA 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: The kit removes most DNA residue and the purified RNA generally requires no DNase I treatment. If the downstream application is very sensitive to trace DNA, treat the RNA with DNase I (CYRN45) or add an on-column DNase digestion step during extraction (CYRN34).

❖ Procedure

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

1. Place 550 µl of Lysis Buffer FEA in a 1.5 ml centrifuge tube and add 55 µl of PlantAid.

2. Grind plant or fungal tissue in liquid nitrogen to a fine powder, transfer 50-100 mg of powder to the FEA tube (with PlantAid) and vortex vigorously for 30 seconds to mix and lyse completely. Centrifuge at 13,000 rpm for 5-10 minutes. Adjust the sample amount as needed; for example, high-water-content fruit can be increased. PlantAid helps remove polysaccharide and polyphenol impurities; for ordinary plants such as rice leaves it may be omitted, and in some cases omitting PlantAid may increase RNA yield and success rate.

3. Immediately take about 500 µl of the supernatant onto a DNA-clear/RNA-bind universal column placed in a collection tube and centrifuge at 13,000 rpm for 30 seconds. Discard the column and keep the filtrate, which contains the RNA. The supernatant volume can be adjusted as needed.

4. Add 0.5 volume of absolute ethanol (about 250 µl, adjusted to the actual supernatant volume) to the filtrate and mix by pipetting. Cloudiness or flocculation is normal; load the mixture, including any flocculent material, onto the universal column and continue.

5. Immediately transfer the mixture to a new universal column placed in a collection tube. Leave for 1 minute, centrifuge at 13,000 rpm for 30 seconds and discard the flow-through. Return the column to the collection tube. The column capacity is 750 µl; if the mixture exceeds this volume, load it in several portions.

6. 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.

7. Add 500 µl of Wash Buffer RW (check that ethanol has been added), centrifuge at 13,000 rpm for 15 seconds and discard the flow-through.

8. Repeat step 7 once.

9. Return the universal column to the collection tube and centrifuge at 13,000 rpm for 2 minutes. This empty centrifugation removes 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 ddH2O 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 ddH2O to 90 °C or reload the first eluate onto the column for a second elution, which increases concentration by about 10%.

11. The extracted RNA can be used directly in downstream applications or stored at -85 to -65 °C.

❖ Appendix: Rapid protocol

For species with low DNA content such as cotton, tomato and oilseed rape, the filtration step (step 3) can be omitted and the following rapid protocol used. Before first use, add the indicated amount of absolute ethanol to Wash Buffer RW.

1. Place 550 µl of Lysis Buffer FEA in a 1.5 ml centrifuge tube and add 55 µl of PlantAid.

2. Grind plant or fungal tissue in liquid nitrogen to a fine powder, transfer 50-100 mg of powder to the FEA tube (with PlantAid) and vortex vigorously for 30 seconds. Centrifuge at 13,000 rpm for 5-10 minutes and transfer about 500 µl of the supernatant to a new tube. Adjust the sample amount as needed; PlantAid may be omitted for ordinary plants, and omitting it may increase yield in some cases.

3. Add 0.5 volume of absolute ethanol (about 250 µl, adjusted to the actual supernatant volume) to the tube and mix by pipetting. Cloudiness or flocculation is normal; load the mixture, including any flocculent material, onto the universal column.

4. Continue with steps 5-11 of the standard procedure.

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