CYRN33-CHANGYU PLANTpure Universal Plant RNA Kit

Catalog number	Package
CYRN3301	50 preps
CYRN3302	100 preps

Storage and shipping: 15-25 °C, except TRIpure Reagent (store at 4 °C protected from light). Stable for 12 months. TRIpure Reagent can be shipped at room temperature, stored long-term at 4 °C in the dark, and kept at room temperature for 3 months without loss of quality. Keep all solutions tightly capped after use.

Kit components

Component	50 preps (CYRN3301)	100 preps (CYRN3302)
TRIpure Reagent	50 ml (4 °C, dark)	100 ml (4 °C, dark)
Deproteinization Buffer PE	16 ml	32 ml (add ethanol before first use)
Wash Buffer RW	10 ml	25 ml (add ethanol before first use)
RNase-free H2O	5 ml	10 ml
RNase-free spin columns RA	50	100
Collection tubes (2 ml)	50	100

Description

An improved guanidinium thiocyanate/phenol one-step method (TRIzol method) lyses cells and inactivates RNases. Total RNA then binds selectively to the silica membrane of the spin column under high-ionic-strength conditions. Rapid wash and centrifugation steps with Deproteinization Buffer and Wash Buffer remove metabolites, proteins and other impurities, and purified RNA is eluted in low-salt RNase-free water.

Features

The silica membrane is specially selected; variation in binding capacity between columns is minimal and reproducibility is high.

Combines the stability and purity of the guanidinium thiocyanate/phenol one-step reagent with the convenience of a spin column. No isopropanol precipitation or ethanol washing is required, and RNA is eluted directly from the column, avoiding the poor solubility of over-dried pellets.

TRIpure Reagent corresponds to Thermo Fisher/Invitrogen TRIzol (catalog number 15596026) and effectively eliminates genomic contamination; performance is consistent with the imported product.

Repeated wash steps remove protein and give higher RNA purity.

Effectively reduces the 5S rRNA content of total RNA and improves purity.

Notes before starting

Note: The kit inhibits RNases very effectively; unless otherwise stated, all centrifugation steps can be performed at room temperature.

Note: TRIpure Reagent and Deproteinization Buffer PE contain irritant compounds. Wear gloves and avoid contact with skin, eyes and clothing. Rinse affected areas with plenty of water or saline.

Note: For environmental reasons the kit does not contain chloroform. Chloroform must be supplied by the user (or the CYRN66 chloroform substitute can be purchased).

Note: Conventional agarose gel electrophoresis and formamide-denaturing gel electrophoresis can both be used to assess RNA quality. Good RNA shows two prominent rRNA bands after denaturing electrophoresis at about 5 kb (28S) and 2 kb (18S) (on ordinary agarose gels 28S and 18S appear at about 2 kb and 1 kb, and their positions vary with RNA secondary structure), with a brightness ratio of about 2:1. Bands at about 0.1 kb and 0.3 kb (5S and tRNA) may also be visible. Four or five bands can be normal in some species or plant tissues, and discontinuous high-molecular-weight bands between 7 kb and 15 kb may appear if RNA precursors, heterogeneous nuclear RNA or small nuclear RNA are extracted.

Note: When measuring the A260/A280 ratio, dilute the RNA sample with TE (pH 8) if dilution is needed. Dilution with water lowers ionic strength and pH, which raises A280 and lowers the ratio.

Note: After homogenization in TRIpure and before adding chloroform, the sample can be stored at -60 to -70 °C for more than one month.

Note: This kit is intended for plant samples that can be extracted with TRIzol. For plant samples rich in polysaccharides and polyphenols where extraction is poor, use the EASYspin plant RNA kits (CYRN38 or CYRN53).

❖ Procedure

Before first use, add absolute ethanol to Wash Buffer RW and Deproteinization Buffer PE as indicated on the labels, mix well and tick the boxes to avoid adding ethanol twice.

1. Transfer 1 ml of TRIpure Reagent to a 1.5 ml centrifuge tube.

2. Grind plant tissue in liquid nitrogen to a fine powder, transfer 60-100 mg of powder to the TRIpure tube and shake vigorously by hand for 20 seconds (manual or electric homogenization can increase yield).

3. Vortex vigorously and incubate at 15-30 °C for 5 minutes to allow complete dissociation of nucleoprotein complexes.

4. Optional (generally unnecessary): centrifuge at 4 °C at 12,000 rpm for 10 minutes and carefully transfer the supernatant to a new 1.5 ml tube. This additional separation step may be needed for samples rich in polysaccharides or for plant tubers.

5. Add 0.2 ml of chloroform per 1 ml of TRIpure. Close the tube tightly, shake vigorously for 15 seconds and incubate at room temperature for 3 minutes. If chloroform is unavailable, the CYRN66 chloroform substitute can be purchased.

6. Centrifuge at 4 °C at 12,000 rpm for 10 minutes. The sample separates into three layers: lower organic phase, interphase and upper colorless aqueous phase containing RNA. The aqueous phase is about 50% of the TRIpure volume. Carefully transfer the aqueous phase to a new tube without touching the interphase, and record its volume.

7. Add 0.5 volume (half the aqueous volume) of absolute ethanol and mix (a precipitate may form). Transfer the solution, together with any precipitate, to spin column RA placed in a collection tube (if the entire mixture does not fit, load it in two portions). Centrifuge at room temperature (all subsequent steps are at room temperature) at 12,000 rpm for 30 seconds, discard the flow-through and return the column to the collection tube.

8. Add 500 µl of Deproteinization Buffer PE (check that ethanol has been added), centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

9. Add 500 µl of Wash Buffer RW (check that ethanol has been added), centrifuge at 12,000 rpm for 15 seconds and discard the flow-through.

10. Repeat step 9 once.

11. Return column RA to the empty collection tube and centrifuge at 12,000 rpm for 2 minutes to remove residual wash buffer, since residual ethanol can inhibit downstream reactions.

12. Transfer column RA to a new 1.5 ml centrifuge tube. Apply 50-80 µl of RNase-free ddH2O to the centre of the membrane, leave for 2 minutes and centrifuge at 12,000 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 70-90 °C before elution or reload the first eluate onto the column for a second elution.

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