CYRN12-CHANGYU PLANTeasy Plant RNA Reagent

Catalog number	Package
CYRN1201	20 ml
CYRN1202	100 ml

Storage and shipping: shipped at room temperature; store at 4 °C. Stable for 6 months.

Applications

For extraction of total RNA from plant tissues rich in polysaccharides and polyphenols.

Description

PLANTeasy reagent extracts highly pure total RNA from plant tissues, especially tissues rich in polyphenols or starch, such as potato tuber, pine needles or young shoots, and tomato leaves. Each 100 ml of reagent processes 200 samples of 100 mg tissue or 4 samples of 5 g tissue.

Materials to be supplied by the user

Liquid nitrogen, mortar, RNase-free centrifuge tubes, 5 M NaCl, chloroform, isopropanol, 75% ethanol and RNase-free water.

Sample preparation

a. Pre-chill RNase-free centrifuge tubes on dry ice and add the ground frozen tissue.

b. Grind fresh plant tissue to a powder in liquid nitrogen; dry seeds can be ground at room temperature.

c. Keep processed plant material at -70 °C until it is suspended in the extraction reagent.

❖ Procedure, small scale (less than 0.1 g sample)

1. Take no more than 0.1 g of frozen ground plant tissue, add 0.5 ml of extraction reagent (4 °C) and vortex until thoroughly mixed.

2. Leave at room temperature for 5 minutes. Lay the tube flat so that the surface area is maximal.

3. Centrifuge at room temperature at 12,000 rpm for 1 minute and transfer the supernatant to a new RNase-free tube.

4. Add 0.1 ml of 5 M NaCl and mix gently, then add 0.3 ml of chloroform and mix by inversion.

5. Centrifuge at 4 °C at 12,000 rpm for 10 minutes and transfer the upper aqueous phase to a new RNase-free tube. For plants rich in polysaccharides and polyphenols, re-extract once with another 0.3 ml of chloroform.

6. Add an equal volume of isopropanol, mix and leave at room temperature for 10 minutes.

7. Centrifuge at 4 °C at 12,000 rpm for 10 minutes. Discard the supernatant without dislodging the pellet and add 1 ml of 75% ethanol. The pellet may be hard to see; handle carefully.

8. Centrifuge at 4 °C at 5,000 rpm for 3 minutes. Pour off the liquid without dislodging the pellet. Collect any remaining liquid by a brief additional centrifugation and remove it with a pipette tip, then air-dry at room temperature for 2-3 minutes.

9. Add 50 µl of RNase-free water and pipette repeatedly to dissolve the RNA. If a flocculent material remains, centrifuge at room temperature at 12,000 rpm for 1 minute and transfer the supernatant to a clean RNase-free tube. Store at -70 °C.

❖ Procedure, large scale (more than 0.1 g up to 5 g sample)

1. For each 1 g of frozen ground plant tissue, add 5 ml of reagent and vortex until thoroughly mixed.

2. Leave at room temperature for 5 minutes. Lay the tube flat so that the surface area is maximal.

3. Centrifuge at 4 °C at 10,000 rpm for 1 minute and transfer the supernatant to a new RNase-free tube. For each 10 ml of supernatant, add 2 ml of 5 M NaCl and mix.

4. For each 10 ml of supernatant, add 6 ml of chloroform and mix by inversion.

5. Centrifuge at 4 °C at 10,000 rpm for 15 minutes and transfer the upper aqueous phase to a new RNase-free tube. For plants rich in polysaccharides and polyphenols, re-extract once with another 6 ml of chloroform.

6. Measure the aqueous phase volume and add 0.9 volume of isopropanol. Mix and leave at room temperature for 10 minutes.

7. Centrifuge at 4 °C at 10,000 rpm for 15 minutes. Discard the supernatant without dislodging the pellet and add 5-10 ml of 75% ethanol.

8. Centrifuge at 4 °C at 5,000 rpm for 5 minutes. Carefully pour off the liquid without dislodging the pellet. Collect any remaining liquid by a brief additional centrifugation, remove it with a pipette tip and air-dry at room temperature for 3-5 minutes.

9. Add RNase-free water according to the pellet size and pipette repeatedly to dissolve the RNA (for example 200 μl per 1 g of leaf). If a flocculent material remains, centrifuge at room temperature at 12,000 rpm for 1 minute and transfer the supernatant to a clean RNase-free tube. Store at -70 °C.

Troubleshooting

Problem	Comments and suggestions
Low RNA yield	Insufficient grinding: grind the sample thoroughly to a powder. Incomplete mixing with the reagent: vortex thoroughly. Low RNA content in the sample: add 1 μl of glycogen (20 μg/μl) per ml of extraction reagent to aid precipitation. Nylon membrane clogging: filter through gauze.
RNA degradation	Improper sample storage: store samples at -70 °C after collection or for long-term storage. Sample thawed before adding reagent: keep samples at -70 °C until they are suspended in the reagent.
Low A260/A280 ratio	RNA dissolved in water: dissolve RNA in 10 mM Tris-HCl (pH 7.5) for UV measurement.
Odor from waste	The reagent contains β-mercaptoethanol: dilute the waste with water, add a few ml of 3% hydrogen peroxide, leave overnight and adjust the pH with sodium bicarbonate to reduce acidity.

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