Concert™ Plant RNA Reagent

Catalog no. 12322-012

General Information

Description

Invitrogen's Concert™ Plant RNA Reagent is a proprietary RNA isolation reagent that allows isolation of high quality total RNA from plant tissues, especially those rich in polyphenolics or starch. Use of the Concert™ Plant RNA Reagent results in high yields of high quality total RNA from plant tissues such as potato tuber, white pine (needles or spring shoot), blue spruce needles, and tomato leaves.

Shipping and Storage

Concert™ Plant RNA Reagent is shipped at room temperature. Upon receipt, store the reagent at +4°C. Product is guaranteed for 6 months from date of shipment, if stored properly.

Contents

Volume supplied: 100 ml

Amount supplied is sufficient for 200 reactions using 100 mg of tissue or 4 reactions using 5 g of tissue.

Concert™ Plant RNA Reagent contains β-mercaptoethanol and sodium azide. Use the reagent in a fume hood. Wear gloves and eye protection when handling the reagent and solutions containing the reagent.

Quality Control

Concert™ Plant RNA Reagent is functionally assayed by treating 20 µg of RNA Ladder (0.24-9.5 kb) with the reagent. After analysis by agarose gel electrophoresis, all bands should be recovered with no evidence of degradation when compared to the control.

Patent

Patent pending.

Continued on next page

Using the Plant RNA Reagent

Two different procedures are available for isolating RNA from plants. Use the table below to select the procedure appropriate for your sample.

Sample Size	Procedure	Page
≤ 0.1 g	Small-Scale Isolation	3
> 0.1 g to 5 g	Large-Scale Isolation	5

Handling RNA

Follow the precautions listed below to avoid contaminating your sample with RNases:

- Always wear disposable gloves and change them frequently
- Use good microbiological technique to avoid contamination
- Use sterile, disposable plasticware
- Use pipettes specially reserved for RNA work
- Use aerosol-resistant pipette tips to reduce sample-to-sample contamination or reagent contamination.
- Treat non-disposable items with RNase *AWAY*™ or similar product to remove RNase contamination
- Bake glassware at 150°C for 4 hours
- Soak non-disposable plasticware in 0.5 M NaOH for 10 minutes, rinse thoroughly with RNase-free water
- Prepare RNase-free water by drawing the water into RNase-free containers, adding diethylpyrocarbonate (DEPC) to a final concentration of 0.01% (v / v). Let stand overnight and autoclave. Use this water to prepare RNase-free solutions.

Small Scale RNA Isolation

Introduction

Use this procedure to isolate RNA from up to 0.1 g of plant tissue.

Additional Materials Needed

Be sure to have the following reagents and equipment prepared before starting the procedure.

- Liquid nitrogen
- Mortar and pestle
- RNase-free microcentrifuge tubes
- 5 M NaCl (RNase-free, Catalog no. 24740-011)
- Chloroform
- Isopropyl alcohol
- 75% ethanol
- RNase-free water (Catalog no. 15230-170, 15230-147, 10977-015)

Sample Preparation

1. Cool RNase-free microcentrifuge tubes in dry ice before placing frozen tissue in them.
2. To prepare any fresh plant tissue, grind the tissue to a powder in liquid nitrogen. For dry seed, grind at room temperature.
3. Store all ground plant material at -70°C. Frozen tissue must remain frozen at -70°C prior to extraction with Plant RNA Reagent. Accidental thawing may result in RNA degradation.

Procedure

1. Add 0.5 ml of cold (+4°C) Plant RNA Reagent for up to 0.1 gram of frozen, ground tissue. Mix by brief vortexing or flicking the bottom of the tube until the sample is thoroughly re-suspended.
2. Incubate the tube for 5 minutes at room temperature. **Note**: Lay the tube down horizontally to maximize surface area during RNA extraction.
3. Clarify the solution by centrifuging for 2 minutes at 12,000 × *g* in a microcentrifuge at room temperature. Transfer the supernatant to an RNase-free tube.
4. Add 0.1 ml of 5 M NaCl to the clarified extract and tap tube to mix.
5. Add 0.3 ml of chloroform. Mix thoroughly by inversion.
6. Centrifuge the sample at +4°C for 10 minutes at 12,000 × *g* to separate the phases. Transfer the top, aqueous phase to an RNase-free tube.
7. Add to the aqueous phase an equal volume of isopropyl alcohol. Mix and let stand at room temperature for 10 minutes.
8. Centrifuge the sample at +4°C for 10 minutes at 12,000 × *g*.

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Procedure, continued

8. Decant the supernatant, taking care not to lose the pellet and add 1 ml of 75% ethanol to the pellet. **Note**: Pellet may be difficult to see.
9. Centrifuge at room temperature for 1 minute at 12,000 × g. Decant the liquid carefully, taking care not to lose the pellet. Briefly centrifuge to collect the residual liquid and remove it with a pipet.
10. Add 10-30 µl RNase-free water to dissolve the RNA. Pipet the water up and down over the pellet to dissolve the RNA. If any cloudiness is observed, centrifuge the solution at room temperature for 1 minute at 12,000 × g and transfer the supernatant to a fresh tube. Store at -70°C.

Large-Scale RNA Isolation

Introduction

Use this procedure to isolate RNA from > 0.1 g to 5 g of plant tissue.

Additional Materials Needed

Be sure to have the following reagents and equipment prepared before starting the procedure.

- Liquid nitrogen
- Mortar and pestle
- RNase-free 15 or 50 ml tubes (15 ml tubes for >0.1 to 1 g tissue; 50 ml for 1-5 g tissue)
- RNase-free 1.5 ml microcentrifuge tubes
- 5 M NaCl (RNase-free, Catalog no. 24740-011)
- Chloroform
- Isopropyl alcohol
- 75% ethanol
- RNase-free water (Catalog no. 15230-170, 15230-147, 10977-015)
- 100 µm nylon sieve (For 50 ml tube only; Falcon, Catalog no. 352360) or muslin (5 × 5 cm for 15 ml tubes or 8 × 8 for 50 ml tubes; Crosby and Baker, Springfield, MA)

Sample Preparation

1. Cool RNase-free polypropylene tubes in dry ice before placing frozen tissue in them.
2. To prepare any fresh plant tissue, grind the tissue to a powder in liquid nitrogen. For dry seed, grind at room temperature.
3. Store all ground plant material at -70°C. Frozen tissue must remain frozen at -70°C prior to extraction with Plant RNA Reagent. Accidental thawing may result in RNA degradation.

Procedure

1. Add 5 ml of cold (+4°C) Plant RNA Reagent per 1.0 gram of frozen, ground tissue. Mix by vortexing and tapping the bottom of the tube until the sample is thoroughly re-suspended.
2. Incubate the tube for 5 minutes at room temperature. **Note**: Lay the tube on its side during incubation to maximize surface area during RNA extraction.
3. Centrifuge the mixture at +4°C for 5 minutes at 2,600 × *g* in a tabletop centrifuge. Pass the supernatant through a 100 µm nylon sieve or muslin and collect the filtrate in an RNase-free tube.
4. Per 10 ml of clarified supernatant, add 2 ml of 5 M NaCl and mix the sample.
5. Per 10 ml of the original clarified supernatant, add 6 ml of chloroform to the solution in Step 3.

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Procedure, continued

6. Centrifuge the sample at +4°C for 30 minutes at 2,600 × *g* to separate the phases. Transfer the top, aqueous phase to an RNase-free tube.
7. Measure the volume of the aqueous phase and add 0.9 volume of isopropyl alcohol. Mix and let stand at room temperature for 10 minutes.
8. Centrifuge the sample at +4°C for 30 minutes at 2,600 × *g* and decant the supernatant, taking care not to lose the pellet.
9. Add 5 to 10 ml of 75% ethanol to the pellet and centrifuge at +4°C for 5 minutes at 2,600 × *g*.
10. Decant the liquid carefully, taking care not to lose the pellet. Briefly centrifuge to collect the residual liquid, and carefully remove it with a pipet.
11. Add RNase-free water to dissolve the RNA (e.g., 250 µl of water for 5 g of corn seed or 500 µl for 5 g corn leaves). Pipet the water up and down over the pellet to dissolve the RNA.
12. Transfer the RNA solution to an RNase-free microcentrifuge tube. If any cloudiness is observed, centrifuge the solution at room temperature for 1 minute at 12,000 × *g* and transfer the supernatant to a fresh tube. Store at -70°C.

Troubleshooting

Help Table

Use the table below to troubleshoot any problem.

Problem	Reason	Solution
Low RNA Yield	Sample ground too coarsely	Grind sample to a fine powder. Grind seeds in a coffee mill to a fine powder.
	Sample incompletely dispersed in Plant RNA Reagent	Vortex to thoroughly resuspend the plant tissue (ensure that it is off the bottom of the tube) and reduce the size of clumps.
	Sample has low endogenous levels of RNA	Add 1 µl of 20 µg/µl glycogen per ml of clarified RNA extract to aid RNA precipitation.
	Nylon sieve clogged	Clarify samples through muslin.
RNA Degraded	Sample stored improperly after harvesting	Store samples at -70°C after harvesting and for long-term storage.
	Sample allowed to thaw before extracting with Plant RNA Reagent	Keep sample at -70°C until Plant RNA Reagent is added and the powder is dispersed in the reagent.
Low $A_{260/280}$ ratio	RNA was diluted with water	Dilute RNA in 10 mM Tris-HCl (pH 7.5) for UV determination.
Tissue Debris Waste has a Stench.	Plant RNA Reagent contains 2-mercaptoethanol	Place waste tissue debris in a beaker, dilute with water and add a few milliliters of 3% hydrogen peroxide. Let stand overnight, loosely covered. Adjust pH to reduce acidity with sodium bicarbonate. Dispose of liquid and solid waste material.

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
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Once connected to the Internet, launch your web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

MSDS Requests

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1. On the home page, go to the left-hand column under 'Technical Resources' and select 'MSDS Requests'.
2. Follow instructions on the page and fill out all the required fields.
3. To request additional MSDSs, click the 'Add Another' button.
4. All requests will be faxed unless another method is selected.
5. When you are finished entering information, click the 'Submit' button. Your MSDS will be sent within 24 hours.

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In the event of an emergency, customers of Invitrogen can call the 3E Company, 24 hours a day, 7 days a week for disposal or spill information. The 3E Company can also connect the customer with poison control or with the University of California at San Diego Medical Center doctors.

3E Company
Voice: 1-760-602-8700

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Technical Service, continued

Contact us

For more information or technical assistance, please call, write, fax, or email. Additional international offices are listed on our web page (www.invitrogen.com).

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