ambion
by life technologies

PureLink® Plant RNA Reagent

Catalog Number 12322-012 **Size** 100 mL **Store at 2°C to 8°C**

Document Part Number 250432 **Publication Number** MAN0000243 **Revision** 2.0

Description

The PureLink® 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 PureLink® 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.

System Overview

The PureLink® Plant RNA Reagent is added to ground tissue, and the tissue lysed for 5 minutes, before the lysate is clarified by centrifugation. After addition of NaCl and chloroform, phase separation is achieved by centrifugation. The RNA is then precipitated with isopropyl alcohol.

Contents and Storage

The supplied amount (100 mL) of PureLink® Plant RNA Reagent is sufficient reagent to perform 200 RNA isolations from 100 mg plant tissue or 4 isolations from 5 g plant tissue. The product is guaranteed stable for 6 months when stored properly.

Accessory Products

The following products and a wide variety of RT-PCR products available from Life Technologies may be used with the PureLink® Plant RNA Reagent. For more information, visit our website at **www.lifetechnologies.com**.

Product	Quantity	Catalog No.
RNase AWAY®	250 mL	10328-011
UltraPure™ DEPC-treated Water	4 × 100 mL	750024
UltraPure™ DNase/RNase-Free Distilled Water	500 mL	10977-015
UltraPure™ 5 M NaCl	10 L	24740-011
Quant-iT™ RNA Assay Kit	1000 assays	Q-33140
SuperScript™ III First-Strand Synthesis System for RT-PCR	50 rxns	18080-051
Platinum® PCR Supermix	100 rxns	11306-016
Platinum® Quantitative PCR Supermix-UDG (with ROX)	500 rxns	11730-025 (11743-500)

Handling RNA

Follow the guidelines below to avoid contaminating your sample with RNases:

- Always wear disposable gloves and change them frequently
- Use proper microbiological aseptic techniques to avoid contamination
- Use sterile, disposable plastic ware
- 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 products to remove RNase contamination
- Bake glassware at 150°C for 4 hours
- Soak non-disposable plastic ware in 0.5 M NaOH for 10 minutes, rinse thoroughly with RNase-free water

Safety

WARNING! **GENERAL CHEMICAL HANDLING.** For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

The PureLink® Plant RNA Reagent contains 2-mercaptoethanol and sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Using the PureLink® 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	2
>0.1 g to 5 g	Large-Scale Isolation	3

Small Scale RNA Isolation

Use this procedure to purify RNA from ≤0.1 g 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, Cat. no. 24740-011)
- Chloroform
- Isopropyl alcohol
- 75% ethanol
- RNase-free Water (Cat. nos. 10813-012, 750024,750023, 10977-015)

Sample Preparation

1. Cool RNase-free microcentrifuge tubes in dry ice before transferring frozen tissue into the tubes.
2. Grind fresh or frozen plant tissue in liquid nitrogen to a powder using mortar and pestle. Grind dry seed samples at room temperature.
3. Store all ground plant material at –70°C until further use. 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 cold (4°C) PureLink® Plant RNA Reagent to ≤0.1 gram of frozen, ground plant tissue. Mix by briefly vortexing or by flicking the bottom of the tube until the sample is thoroughly resuspended.
2. Incubate the tube for 5 minutes at room temperature.

   Note: Lay the tube down horizontally during incubation to maximize surface area.
3. Clarify the solution by centrifuging at 12,000 × *g* in a microcentrifuge for 2 minutes at room temperature. Transfer the supernatant to a clean RNase-free tube.
4. Add 0.1 mL of 5 M NaCl to the clarified extract. Mix by tapping the tube.
5. Add 0.3 mL chloroform to the sample. Mix thoroughly by inverting the tube.
6. Centrifuge the sample at 12,000 × *g* for 10 minutes at 4°C to separate the phases. Transfer the upper, aqueous phase to a clean 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 12,000 × *g* for 10 minutes at 4°C.
9. 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.

Small Scale RNA Isolation, Continued

10. Centrifuge at 12,000 × *g* for 1 minute at room temperature. Decant the supernatant carefully, taking care not to lose the pellet. Briefly centrifuge to collect the residual liquid and remove it with a pipet.
11. Add 10–30 µL RNase-free Water to the RNA pellet. Pipet the liquid up and down over the pellet to resuspend the RNA. If any cloudiness is observed, centrifuge the solution at room temperature for 1 minute at 12,000 × *g* and transfer the supernatant containing the RNA to a clean tube. Store purified RNA at –70°C.

Large-Scale RNA Isolation

Use this procedure to isolate RNA from 0.1–5 g 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 0.1–1 g plant tissue; 50-mL for 1–5 g plant tissue)
- RNase-free 1.5-mL microcentrifuge tubes
- 5 M NaCl (RNase-free, Cat. no. 24740-011)
- Chloroform
- Isopropyl alcohol
- 75% ethanol
- RNase-free Water (Cat. nos. 15230-170, 15230-147, 10977-015)
- 100 µm nylon mesh (for 50-mL tube only; Falcon, Cat. no. 352360) or muslin (5 × 5 cm for 15-mL tubes or 8 × 8 cm for 50-mL tubes; Crosby and Baker, Springfield, MA)

Sample Preparation

1. Cool RNase-free polypropylene tubes in dry ice before transferring frozen tissue into the tubes.
2. Grind fresh or frozen plant tissue in liquid nitrogen to a powder using mortar and pestle. Grind dry seed samples at room temperature.
3. Store all ground plant material at –70°C until further use. 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 cold (4°C) PureLink® Plant RNA Reagent per 1.0 gram frozen, ground tissue. Mix by vortexing and tapping the bottom of the tube until the sample is thoroughly resuspended.
2. Incubate the tube for 5 minutes at room temperature.

   Note: Lay the tube down horizontally during incubation to maximize surface area.
3. Centrifuge the mixture at 2,600 × *g* in a tabletop centrifuge for 5 minutes at 4°C. 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. Mix by inverting the tube several times.
5. Per 10 mL of the original clarified supernatant, add 6 mL of chloroform to the sample. Mix thoroughly by inverting the tube.
6. Centrifuge the sample at 2,600 × *g* for 30 minutes at 4°C to separate the phases. Transfer the upper, aqueous phase to a clean 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 2,600 × *g* for 30 minutes at 4°C and decant the supernatant, taking care not to lose the pellet.
9. Add 5–10 mL of 75% ethanol to the pellet and centrifuge at 2,600 × *g* for 5 minutes at 4°C.
10. Decant the supernatant 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 the RNA pellet (e.g., 250 µL of water for 5 g of corn seed or 500 µL for 5 g corn leaves). Pipet the liquid up and down over the pellet to resuspend the RNA.
12. Transfer the RNA solution to an RNase-free microcentrifuge tube. If any cloudiness is observed, centrifuge the solution at 12,000 × *g* for 1 minute at room temperature and transfer the supernatant containing the RNA to a clean tube. Store purified RNA at –70°C.

Troubleshooting

Use the table below to troubleshoot any problems you may encounter when using the PureLink® Plant RNA Reagent.

Problem	Cause	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 PureLink® Plant RNA Reagent	Vortex thoroughly to 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 PureLink® Plant RNA Reagent	Keep samples at –70°C until PureLink® 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.	PureLink® 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.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Disclaimer

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.