RNAprep Pure Plant Kit

For purification of total RNA from plant cells and tissues

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RNAprep Pure Plant Kit

(Spin Column)

Cat. no. GDP432

Kit Contents

	Contents	GDP432 (50 preps)
GDP432H	Buffer RL	30 ml
	Buffer RW1	40 ml
	Buffer RW	12 ml
	RNase-Free ddH_2O	15 ml
	RNase-Free Columns CR3 set	50
	RNase-Free Columns CS set	50
	RNase-Free Centrifuge Tubes (1.5ml)	50
	Handbook	1
GRT411	RNase-Free DNase I (1500 U)	1
	Buffer RDD (DNA Digest Buffer)	4 ml
	RNase-Free ddH_2O	1 ml

Note: GDP432H, GRT411 are shipped and packaged separatly.

Storage

RNase-Free DNase I, Buffer RDD and RNase-Free ddH_2O (Tubular) should be stored at 2-8°C for 15 months; Buffer RL/β-mercaptoethanol mix can be stored at 2-8°C for 1 month; others stored at room temperature (15-30°C) for 15 months.

Introduction

RNAprep Pure Plant Kit provides a fast, simple, and cost-effective method for purification of total RNA from plant cells and tissues. The purified RNA is ready for use in downstream applications such as RT-PCR and real-time RT-PCR, microarray, northern blot, dot blot, polyA screening, in vitro transcription, and molecular cloning.

Notes of preventing RNase contamination

1. Change gloves regularly. Bacteria on the skin can result in RNase contamination.
2. Use RNase-Free plastic ware and tips to avoid RNase contamination.
3. RNA can be protected in Buffer RL. But RNA must be stored or applied in RNase-Free plastic or glassware. To wipe off RNase, the glassware can be roasted at 150°C for 4 hours, while plastic can be dipped in 0.5 M NaOH for 10 min, washed by ddH_2O thoroughly and sterilization.
4. Use RNase-Free ddH_2O to confect solution.

RNA Yield

Leaves of Plants (100 mg)	Total RNA Yield (μg)
Arabidopsis Thaliana	~35
Maize	~25
Tomato	~65
Tobacco	~60

Important notes before starting

1. β-Mercaptoethanol (β-ME) must be added to Buffer RL before use. The final concentration of β-ME is 1%. For example, add 10 μl β-ME to 1 ml Buffer RL. Buffer RL containing β-ME can be stored at 2-8°C for 1 month. Buffer RL may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature (15-30°C).
2. RNAprep pure Plant Kit provides the lysis Buffer RL, which contain guanidine thiocyanate that can be used for most samples. For tissues with special secondary metabolites (such as milky endosperm of Maize), guanidine thiocyanate can lead solidification of the sample, which will affect RNA extraction. In this scenario, Tiangen can provide an alternative lysis buffer, Buffer HL, free of charge.

3. Buffer RW is supplied as a concentrate. Before using for the first time, add ethanol (96-100%) as indicated on the bottle to obtain a working solution.
4. Perform all steps and centrifugation steps at room temperature (15-30°C) in a standard micro centrifuge.

Preparation of DNase I stock solution

Dissolve the lyophilized DNase I (1500 U) in 550 µl RNase-Free ddH_2O (Tubular). Mix gently by inverting. Do not vortex. Divide it into single-use aliquots, and store at -30~-15°C for up to 9 months. Thawed aliquots can be stored at 2-8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Protocol

1. Place the 50-100 mg tissue in liquid nitrogen immediately, and grind thoroughly with a mortar and pestle. Add 450 µl Buffer RL **(Ensure that β-ME has been added to Buffer RL before use)** to the maximum of 100 mg tissue powder. Vortex vigorously.

   Note 1: Short time (1-3 min) incubation at 56°C may help to disrupt the tissue. However, do not incubate samples with high starch content at elevated temperatures; otherwise swelling of the sample will occur.

   Note 2: Plants are quite diversified, and RNA contents varied in different growth stage and different tissues, so please use appropriate plant volumes depending on specific conditions.
2. Transfer the lysate to RNase-Free Filter Column CS placed in a 2 ml Collection Tube, and centrifuge for 2-5 min at 12,000 rpm (~13,400 × g). Carefully transfer the supernatant to a new centrifuge tube (not supplied) without disturbing the cell-debris pellet in the Collection Tube. Use only this supernatant in subsequent steps.

   Note: It may be necessary to cut off the end of the pipet tip to facilitate pipetting of the lysate into the spin column.
3. Add 0.5 volume of ethanol (96-100%) to the cleared lysate, and mix immediately by pipetting. Transfer the sample, including any precipitate that may have formed, to RNase-Free Spin Column CR3 placed in a 2 ml Collection Tube. Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.

Note: If there is a loss in the volume of the supernatant, adjust the amount of ethanol accordingly.

4. Add 350 µl Buffer RW1 to the Spin Column CR3. Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
5. Preparation of DNase I working solution: Add 10 µl DNase I stock solution (see Preparation of DNase I stock solution) to 70 µl Buffer RDD. Mix by gently inverting the tube.
6. Add the DNase I working solution (80 µl) directly to the center of Spin Column CR3, and place on the bench top for 15 min.
7. Add 350 µl Buffer RW1 to the Spin Column CR3. Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
8. Add 500 µl Buffer RW to the Spin Column CR3 **(Ensure that ethanol has been added to Buffer RW before use).** Close the lid gently, place in room temperature for 2 min and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through.
9. Repeat step 8.
10. Centrifuge for 2 min at 12,000 rpm (~13,400 × g) to dry the spin column membrane.

    Note: The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.
11. Place the Spin Column CR3 in a new 1.5 ml Collection Tube (supplied). Add 30-100 µl RNase-Free water directly to the spin column membrane. Close the lid gently, place in room temperature for 2 min and centrifuge for 2 min at 12,000 rpm (~13,400 × g) to elute the RNA.

    Note: Volume of elution buffer should no less than 30 µl, or else it will reduce elution efficiency. Purified RNA may be stored at –70°C.