EasyPrep Plant RNAprep Purification Kit

For purification of total RNA from plant cells and tissues

Contents

Introduction

The EasyPrep Plant RNAprep Purification Kit provides a fast, simple, and cost-effective method for the purification of total RNA from plant cells and tissues. The purified RNA is ready for use in downstream applications such as reverse transcription polymerase chain reaction (RT-PCR) and real-time RT-PCR, microarray, Northern blotting, dot blotting, polyA screening, in vitro transcription, and molecular cloning.

Notes for preventing RNase contamination

1. Change gloves regularly. Bacteria on the skin can result in RNase contamination.
2. Use RNase-free plastic and tips to avoid cross-contamination.
3. RNA can be protected in Buffer RBL. However, RNA must be stored or RNA experiments must be conducted in RNase-free plastic or glassware. To remove/degrade RNase, glassware can be heated at 150℃ for 4 h, and plastic can be immersed in 0.5 M NaOH for 10 min, washed with RNase-free ddH_2O thoroughly, and sterilized.
4. Use RNase-free ddH_2O to confect solution.

Yield of total RNA purified using the EasyPrep Plant RNAprep Purification Kit

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

Important points before starting the purification

1. β-Mercaptoethanol (β-ME) must be added to Buffer RBL before use. The final concentration of β-ME is 1%. For example, 10 μL β-ME is added to 1 mL Buffer RBL. Buffer RBL containing β-ME can be stored at 4°C for 1 month. Buffer RBL may form a precipitate upon storage. If necessary, it can be redissolved by warming, and then, the buffer can be stored at room temperature (15°C–25°C).
2. In the kit, the lysis buffer of Buffer RBL is provided, which contains guanidine thiocyanate; it can be used for most samples. For tissues with special secondary metabolites (such as the milky endosperm of maize), guanidine thiocyanate can lead to the solidification of the sample, which will affect RNA extraction. In this scenario, TOOLS provides an alternative lysis buffer, Buffer HL.
3. Buffer RBW is supplied as a concentrate. Before use, add 48 mL ethanol (96%–100%), as indicated on the bottle, to obtain a working solution.
4. Perform all steps in RT; if not indicated, centrifugation steps should be performed at 20°C–25°C in a standard microcentrifuge.

Preparation of DNase I stock solution

Dissolve the lyophilized DNase I (1500 U) in 550 µL RNase-free ddH_2O. Mix gently by inverting. Do not vortex. Divide it into single-use aliquots, and store at –20°C for up to 9 months. Thawed aliquots can be stored at 2°C–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Kit Contents

Contents	DPT-BD32 (50 preps)
Buffer RBL	30 mL
Buffer DBRW1	40 mL
Buffer RBW	12 mL
DNase I（1500 U）	1
Buffer RBDD	4 mL
RNase-free ddH_2O（Tubular）	1 mL
RNase-free ddH_2O（Bottled）	15 mL
RNase-free Spin Columns CR3	50
RNase-free Filtration Columns CS	50
RNase-free Collection Tubes（1.5 ml）	50

Storage

RNase-free DNase I, Buffer RBDD, and RNase-free ddH_2O (tubular) should be stored at 2°C–8°C. The Buffer RBL/β-mercaptoethanol mix can be stored at 4℃ for 1 month; the remaining reagents are stored at room temperature (15°C–25°C).

Protocol

1. Place 50–100 mg tissue in liquid nitrogen immediately and grind thoroughly with a mortar and pestle. Add 450 µL Buffer RBL (ensure that β-ME is added to Buffer RBL before use) to a maximum weight of 100-mg tissue powder. Vortex vigorously.

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

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 microcentrifuge 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 pipette 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 through 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.
4. Add 350 µL Buffer DBRW1 to the CR3 spin column. 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 RBDD. Mix by gently inverting the tube.
6. Add the DNase I working solution (80 µL) directly to the center of the CR3 spin column, and place it on the bench top (20°C–30°C) for 15 min.
7. Add 350 µL Buffer DBRW1 to the CR3 spin column. 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 RBW to the CR3 spin column (ensure that ethanol is added to Buffer RBW before use). Close the lid gently, incubate at 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 protocol dries the spin column membrane, ensuring that all ethanol is removed during RNA elution. Residual ethanol may interfere with downstream reactions.

11. Place the CR3 spin column in a new 1.5-mL collection tube (supplied). Add 50–100 µL RNase-free water directly to the spin column membrane. Close the lid gently, incubate at room temperature for 2 min, and centrifuge for 2 min at 12,000 rpm (~13,400 × g) to elute the RNA.

Note: If elution is performed in small volumes (<50 µL), the elution buffer must be dispensed onto the center of the membrane for complete elution of bound RNA. Purified RNA may be stored at −70°C.