

RNAprep Pure Kit (For Plant)

For purification of total RNA from
plant cells and tissues



RNAprep Pure Kit (For Plant)

(Spin Column)

Cat. no. DP432

Kit Contents

Contents	DP432 50 preps
Buffer RL	30 ml
Buffer RW1	40 ml
Buffer RW	12 ml
RNase-free ddH ₂ O	15 ml
RNase-free Spin Column CR3 in a 2 ml Collection Tube	50
RNase-free Filtration Column CS in a 2 ml Collection Tube	50
DNase I (1500 U)	1
Buffer RDD	4 ml
Rnase-Free ddH ₂ O	1 ml
RNase-Free Centrifuge Tubes 1.5 ml	50
Handbook	1

Storage

RNase-Free DNase I, Buffer RDD & RNase-Free ddH₂O (Tubular) should be stored at 2-8°C; Buffer RL/ β -mercaptoethanol mix can be stored at 4°C for 1 month; others stored at room temperature (15-25°C).

Introduction

RNAprep Pure Kit (For Plant) 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 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 ddH₂O thoroughly and sterilization.
4. Use RNase-Free ddH₂O to confect solution.
5. RNA Yield

Leaves of Plants (100 mg)	Total RNA Yield (μg)
Arabidopsis thaliana	~35
Corn	~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 4°C for 1 month. Buffer RL may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature (15-25°C).

2. RNAPrep pure Kit (For Plant) 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 (20-25°C) in a standard micro centrifuge.

Preparation of DNase I stock solution

Dissolve the lyophilized DNase I (1500U) in 550 μ l RNase-Free ddH₂O (Tubular). 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 2-8°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 μ l Buffer RL (Ensure that β -ME is added to Buffer RL before use) to the maximum of 100 mg tissue powder. Vortex vigorously.

Note: 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.

2. Transfer the lysate to RNase-Free Filter Columns CS placed in a 2 ml collection tube, and centrifuge for 2-5 min at 12,000 rpm ($\sim 13,400 \times g$). 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 ($\sim 13,400 \times g$). Discard the flow-through.
4. Add 350 μ l Buffer RW1 to the spin column CR3. Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm ($\sim 13,400 \times g$). Discard the flow-through.
5. Preparation of DNase I working solution: Add 10 μ l DNase I stock solution (see Preparation of DNase I stock solution) to 70 μ l Buffer RDD. Mix by gently inverting the tube.
6. Add the DNase I working solution (80 μ l) directly to the center of spin column CR3, and place on the bench top (20-30°C) for 15 min.
7. Add 350 μ l Buffer RW1 to the spin column CR3. Close the lid gently, and centrifuge for 30-60 s at 12,000 rpm ($\sim 13,400 \times g$). Discard the flow-through.
8. Add 500 μ l Buffer RW to the CR3 spin column (Ensure that

ethanol is added to Buffer RW before use). Close the lid gently, place in room temperature for 2 min and centrifuge for 30-60s at 12,000 rpm ($\sim 13,400 \times g$). Discard the flow-through.

9. Repeat step 8.
10. Centrifuge for 2 min at 12,000 rpm ($\sim 13,400 \times g$) 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 50-100 μ l RNase-Free water directly to the spin column membrane. Close the lid gently, place in room temperature for 2min and centrifuge for 2 min at 12,000 rpm ($\sim 13,400 \times g$) to elute the RNA.

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

Ordering Information

Real-time PCR

Product	Size	Cat. no.
SuperReal PreMix (SYBR Green)	50 μ l \times 50 rxn	FP204-01
	50 μ l \times 200 rxn	FP204-02
Quant One Step qRT-PCR (SYBR Green) Kit	50 μ l \times 50 rxn	FP303-01

Reverse Transcription

Product	Size	Cat. no.
Quantscript RT Kit	20 μ l \times 25 rxn	KR103-03
	20 μ l \times 100 rxn	KR103-04
TIANScript RT Kit	25 rxn	KR104-01
	100 rxn	KR104-02