

# RNAprep Pure Plant Plus Kit

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For purification of total RNA from  
polysaccharides & polyphenolics-rich  
plant)

# RNAprep Pure Plant Plus Kit

(Spin Column)

Cat. no. GDP441

## Kit Contents

	Contents	GDP441 50 preps
GDP441H	Buffer SL	30 ml
	Buffer RW1	40 ml
	Buffer RW	12 ml
	RNase-Free ddH <sub>2</sub> O	15 ml
	RNase-Free Columns CR3 set	50
	RNase-Free Columns CS set	50
	RNase-Free Centrifuge Tubes (1.5 ml)	50
	Handbook	1
GRT411	RNase-Free DNase I (1500 U)	1
	Buffer RDD (DNA Digest Buffer)	4 ml
	RNase-Free ddH <sub>2</sub> O	1 ml

**Note:** GDP441H, GRT411 are shipped and packaged separately.

## Storage

RNase-Free DNase I, Buffer RDD and RNase-Free ddH<sub>2</sub>O should be stored at 2-8°C for 15 months; Buffer SL/ $\beta$ -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 Plus Kit provides a fast, simple, and cost-effective method for purification of total RNA from plant cells and tissues, especially from plant tissues rich in polysaccharides, polyphenolics and starch, such as cotton leaves, mature rice leaves, Arabidopsis thaliana seeds, white pine needles, banana, loquat leaves, potato tubers, apple, pear, watermelon, kiwi fruit, Chinese rose, tobacco, sea-buckthorn, lily, etc. 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 avoiding RNase contamination

1. Wear gloves when handling RNA and all reagents, as skin is a common source of RNases. Change gloves frequently.
2. Use RNase-Free certified, disposable plastic ware and filter tips whenever possible.
3. Buffer SL could protect RNA. But for experiment, RNA should be stored or applied in RNase-Free plastic or glassware.
4. Use RNase-Free ddH<sub>2</sub>O to prepare solution (RNase-Free ddH<sub>2</sub>O: Add 0.1 ml DEPC to 100 ml H<sub>2</sub>O and shake vigorously to bring DEPC into solution. Let the solution stand overnight. Autoclave to remove any trace of DEPC).

## RNA Yield

Sample	Sample amount (mg)	RNA yield (μg)
Banana	100	3-5
Watermelon	100	1.5-2.4
Apple	100	1.2-2
Pear	100	1.2-2
Sweet potato	100	5.5-9
Potato	100	6-10
White pine needles	100	15-20
Cotton leaves	100	20-25
Chinese rose leaves	100	20-25
Alfalfa leaves	100	8-10
Rice leaves	100	20-25

### Important notes before starting

1. Add  $\beta$ -Mercaptoethanol ( $\beta$ -ME) to Buffer SL before use. The final concentration of  $\beta$ -ME is 5%. For example, add 25  $\mu$ l  $\beta$ -ME to 475  $\mu$ l Buffer SL. Buffer SL with  $\beta$ -ME can be stored at 2-8°C for up to one month. Buffer SL may form precipitate upon storage. If necessary, re-dissolve it by warming.
2. RNAprep Pure Plant Plus Kit provides a choice of Buffer HL. Buffer SL can be used for most plant samples. However, for tissues with special secondary metabolites (such as milky endosperm of maize, red bean or wheat seeds) or filamentous fungi, guanidine thiocyanate in Buffer SL may cause sample solidification, which will affect RNA extraction. In this condition, TIANGEN provides an alternative lysis buffer HL.
3. Buffer RW is supplied as a concentrate. Before using for the first time, ethanol (96-100%) should be added as indicated on the bottle to obtain a working solution.

### Preparation of DNase I stock solution

Dissolve the lyophilized DNase I (1500U) in 550  $\mu$ l RNase-Free ddH<sub>2</sub>O. Mix gently by inverting the tube. Do not vortex. Divide it into single-use aliquots, and store at -30~-15°C for up to 9 months. Attention: Thawed aliquots can be stored at 2-8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

### Reagents need to be prepared by Customer

96-100% ethanol,  $\beta$ -Mercaptoethanol

### Protocol

All of the centrifugation steps are performed at room temperature.

1. Place 50-100 mg tissue or fruit pulp in liquid nitrogen immediately, and grind thoroughly with a mortar and pestle. Add 500  $\mu$ l Buffer SL (**Ensure that  $\beta$ -ME has been added to Buffer SL before use**), vortex vigorously.

**Note 1: If the estimated yield <10  $\mu$ g, please starts with 100 mg samples; for starch-rich samples of mature leaves, please increase Buffer SL volume to 700  $\mu$ l.**

**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. Centrifuge for 2 min at 12,000 rpm ( $\sim 13,400 \times g$ ).
3. Transfer the lysate to RNase-Free Filtration Column CS placed in a 2 ml Collection Tube, and centrifuge for 2 min at 12,000 rpm ( $\sim 13,400 \times g$ ). 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.
4. Add 0.4 volume of ethanol (96-100%) to the cleared lysate, and mix immediately by pipetting (probably form precipitation). Transfer the sample, including any precipitate that may have formed, to RNase-Free Spin Column CR3 placed in a 2 ml Collection Tube. Centrifuge for 15 s at 12,000 rpm ( $\sim 13,400 \times g$ ). Discard the flow-through, and put CR3 back into Collection Tube.

**Note: if there is loss of cleared lysate, please adjust ethanol volume accordingly.**

5. Add 350  $\mu$ l Buffer RW1 to the Spin Column CR3. Close the lid gently, and centrifuge for 15 s at 12,000 rpm ( $\sim 13,400 \times g$ ). Discard the flow-through, and put CR3 back into Collection Tube.
6. Preparation of DNase I working solution: Add 10  $\mu$ l DNase I stock solution (see Preparation of DNase I stock solution) to 70  $\mu$ l Buffer RDD. Mix by gently inverting the tube.
7. Add the DNase I working solution (80  $\mu$ l) directly to the center of Spin Column CR3, and place on the bench top (15-30°C) for 15 min.
8. Add 350  $\mu$ l Buffer RW1 to the Spin Column CR3. Close the lid gently, and centrifuge for 15 s at 12,000 rpm ( $\sim 13,400 \times g$ ). Discard the flow-through.
9. Add 500  $\mu$ l Buffer RW to the Spin Column CR3 (**Ensure that ethanol (96-100%) has been added to Buffer RW before use**). Close the lid gently, centrifuge for 15 s at 12,000 rpm ( $\sim 13,400 \times g$ ). Discard the flow-through.
10. Repeat step 9.
11. Centrifuge for 2 min at 12,000 rpm ( $\sim 13,400 \times g$ ) to dry the spin column membrane. Place the Spin Column CR3 in a new 1.5 ml Collection Tube (supplied). Add 30-50  $\mu$ l RNase-Free water directly to the spin column

membrane. Close the lid gently, place in room temperature for 2 min and centrifuge for 1 min at 12,000 rpm ( $\sim 13,400 \times g$ ) to elute the RNA.

**Note:** The elution buffer used should be over 30  $\mu$ l, since small volume has a negative effect on elution efficiency. Purified RNA should be stored at  $-70^{\circ}\text{C}$ . If the anticipated RNA yield is over 30  $\mu$ g, then the RNA derived by centrifugation in step 11 could be add into CR3 again, stand for 2 min at room temperature, then centrifuge for 1 min at 12,000 rpm ( $\sim 13,400 \times g$ ) to get RNA.