TIANGEN®

RNA Easy Fast Plant Tissue Kit

www.tiangen.com/en

RNA Easy Fast Plant Tissue Kit

Cat. No. 4992880

Kit Contents

Contents	4992880 (50 preps)
Buffer SG	40 ml
Buffer RW3	40 ml
Buffer RW	12 ml
Proteinase K	500 µl
RNase-Free ddH_2O	15 ml
gDNA Eraser Column set	50
RNase-Free column CR4 set	50
RNase-Free Centrifuge Tubes (1.5 ml)	50
Handbook	1

Selected Reagent

DNase I (1500 U) (TIANGEN, Cat. no.: 4992232)

Storage

The reagent is stored at room temperature (15-25°C); The selected RNase-Free DNase I is stored at 2-8°C.

Introduction

This product is a RNA easy fast plant tissue kit developed based on the genomic DNA removal technology exclusively by TIANGEN. It does not need toxic reagents such as β-mercaptoethanol or DTT, and is widely suitable for rapid extraction of RNA from various plant samples. RNA extraction can be completed within 30 minutes. The total RNA extracted by this kit has high yield, good purity and no contamination of protein and other impurities. It can be used in many downstream experiments such as RT-PCR, Real Time RT-PCR, Chip analysis, Northern Blot, Dot Blot, PolyA screening, *in vitro* translation, RNase protection analysis and molecular cloning, etc.

Pay attention to the following aspects to prevent RNase contamination:

1. Change new gloves regularly. Bacteria on the skin can result in RNase contamination.
2. Use RNase-Free plastic and tips to avoid cross contamination.
3. RNA will not be degraded by RNase when it is in lysis buffer SG. However, RNase-free plastics and glassware should be used for further treatment. 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 RNase-Free ddH_2O thoroughly, and sterilized.
4. Use RNase-Free ddH_2O to prepare the solutions. (Add DEPC into water in clean glass container to a final concentration of 0.1% (v/v). Incubate overnight and autoclave for 15 min to remove any trace of DEPC.)

Important Notes

1. If the subsequent experiments require high RNA purity, DNase I digestion can be selectively carried out, and see Step 6. DNase I needs to be purchased by yourself, and see the selection reagent for specific models.
2. Ethanol(96%-100%) shall be added to the Buffer RW before the first use as indicated on the bottle.
3. The following operations are performed at room temperature unless specified.

Protocol

Before use, please add ethanol (96%-100%) into Buffer RW as indicated on the bottle.

1. Sample preprocessing

   Grind plant leaves or fruit pulp into powder rapidly in liquid nitrogen, take 30-150 mg sample and add 600 µl Buffer SG and 10 µl Proteinase K, immediately votex to mix thoroughly, then incubate at room temperature for 5 min.

   Notes: As far as possible, the tender part of the leaf sample shall be taken, and the recommended sample weight is 60 mg; For fruit, tuber and petal samples, the recommended weight is 150 mg; For dry seed samples such as red beans, the recommended sample weight is 30 mg, because the lysate is increased to 1 ml volume due to water absorption.

2. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min, and take about 500 µl of supernatant for the following operations.

   Notes: Try not to touch the bottom of the precipitation with the tip to avoid absorbing impurities.

3. The obtained supernatant is added to gDNA Eraser Column (placed in the collection tube) and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec to retain the filtrate.

4. Slowly add 0.5 times volume of ethanol (96%-100%) (about 250 µl) to the above filtrate, and mix well (precipitation may appear), transfer the obtained solution and precipitation together into RNase-Free column CR4 (placed in the collection tube), and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through, and put the column back into the collection tube.

5. If DNase I digestion is not carried out, add 700 µl Buffer RW3 to RNase-Free column CR4, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and put the column back into the collection tube.

6. DNase I digestion (optional): If the subsequent experiments require high RNA purity, DNase I digestion can be selectively carried out.

   1) Add 350 µl Buffer RW3 to RNase-Free Column CR4, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and put the column back into the collection tube.

   2) Preparation of DNase I storage solution:

DNase I dry powder (1500 U) is dissolved in 550 µl RNase-Free ddH_2O, and gently mix. Divide it into single-use aliquots and store at -20°C (for 9 months).

Notes: DNase I storage solution thawed from -20°C is stored at 4°C (can be stored for 6 weeks) and shall not be frozen again.

3) Put 10 µl DNase I storage solution into a new RNase-Free centrifuge tube. Add 70 µl Buffer RDD and gently mix .The resulting solution is the working solution.

4) Add 80 µl DNase I working solution to the center of RNase-Free Column CR4 and incubate at room temperature for 15 min.

5) Add 350 µl Buffer RW3 to RNase-Free Column CR4, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and put the column back into the collection tube.

7. Add 500 µl Buffer RW **(Ensure ethanol has been added before use)** into the RNase-Free Column CR4, and incubate at room temperature for 2 min. Centrifuge at 12,000 rpm (~13,400 × g) for 30-60 sec. Discard the flow-through and put the column back into the collection tube.
8. Repeat Step 7.
9. Centrifuge for 2 min at 12,000 rpm (~13,400 × g) and discard the flow-through. Place the RNase-Free Column CR4 at room temperature for 2 min to thoroughly dry out the residual ethanol.

   Notes: The purpose of this step is to remove the residual ethanol in RNase-Free Column CR4 because it may affect the subsequent RT-PCR and other experiments.

10. Transfer the RNase-Free Column CR4 to a new RNase-Free centrifuge tube. Add 30-100 µl RNase-Free ddH_2O to the center of membrane. Incubate at room temperature for 2 min and centrifuge at 12,000 rpm (~13,400 × g) for 2 min. Collect the eluant.

    Notes: The volume of the elution buffer shall not be smaller than 30 µl, the recovery efficiency will be affected if the volume is too small. The RNA solution shall be stored at -70°C.