PURO
Plant RNA

For purification of total RNA from Plants

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PURO Plant RNA
(Polysaccharides&Polyphenolics-rich)

Cat. no. SC09

Kit Contents

Contents	SC0901 50 preps
Buffer SL	30 ml
Buffer RW1	40 ml
Buffer RW	15 ml
DNase I (1500 U)	1
Buffer RDD	4 ml
Rnase-Free ddH_2O	1 ml
RNase-Free ddH_2O	15 ml
RNase-Free Spin Columns CR3	50
RNase-Free Filtration Columns CS	50
RNase-Free Centrifuge Tubes 1.5 ml	50
RNase-Free Collection Tubes 2 ml	100
Handbook	1

Storage

RNase-Free DNase I, Buffer RDD & RNase-Free ddH_2O (Tubular) should be stored at 2-8°C; Buffer SL/β-mercaptoethanol mix can be stored at 4°C for 1 month; others stored at room temperature (15-25°C).

Introduction

PURO Plant RNA (Polysaccharides & Polyphenolics-rich) 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. SL buffer could protect RNA. But for experiment, RNA should be stored or applied in RNase-Free plastic or glassware.
4. Use RNase-Free ddH_2O to prepare solution (RNase-Free ddH_2O: Add 0.1 ml DEPC to 100 ml H_2O and shake vigorously to bring DEPC into solution. Let the solution stand overnight. Autoclave to remove any trace of DEPC).

RNA Yield

Leaves of Plants (100 mg)	Total RNA Yield (μg)
Cotton leaves	~25
Arabidopsis thaliana seeds	~40
Banana pulp	~5

Important notes before starting

1. Add β-Mercaptoethanol (β-ME) to Buffer SL before use. The final concentration of β-ME is 5%. For example, add 25 µl β-ME to 475 µl Buffer SL. Buffer SL with β-ME can be stored at 4°C for up to one month. Buffer SL may form precipitate upon storage. If necessary, re-dissolve it by warming.
2. PURO Plant RNA (Polysaccharides & Polyphenolics-rich) provides a choice of buffers. 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.
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 µl RNase-Free ddH_2O (Tubular). Mix gently by inverting the tube. Do not vortex. Divide it into single-use aliquots, and store at -20°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.

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 µl Buffer SL (Ensure that β-ME is added to Buffer SL before use), vortex vigorously.

Note 1: If the estimated yield <10 µg, please starts with 100 mg samples; for starch-rich samples of mature leaves, please increase Buffer SL volume to 700 µl.

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 (~13,400 × g).
3. Transfer the lysate to RNase-Free Filter Columns CS placed in a 2 ml collection tube, and centrifuge for 2 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.
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 (~13,400 × g). Discard the flow-through, and put CR3 back into collection column.

   Note: if there is loss of cleared lysate, please adjust ethanol volume accordingly.
5. Add 350 µl Buffer RW1 to the spin column CR3. Close the lid gently, and centrifuge for 15 s at 12,000 rpm (~13,400 × g). Discard the flow-through, and put CR3 back into collection column.
6. 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.

7. Add the DNase I working solution (80 µl) directly to the center of spin column CR3, and place on the bench top (20-30°C) for 15 min.
8. Add 350 µl Buffer RW1 to the spin column CR3. Close the lid gently, and centrifuge for 15 s at 12,000 rpm (~13,400 x g). Discard the flow-through.
9. Add 500 µl Buffer RW to the CR3 spin column (Ensure that ethanol is added to Buffer RW before use). Close the lid gently, centrifuge for 15 s at 12,000 rpm (~13,400 x g). Discard the flow-through.
10. Repeat step 9.
11. Centrifuge for 2 min at 12,000 rpm (~13,400 × g) to dry the spin column membrane. Place the spin column CR3 in a new 1.5 ml collection tube (supplied). Add 30-50 µl 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 (~13,400 × g) to elute the RNA.

    Note: The elution buffer used should be over 30 µl, since small volume has a negative effect on elution efficiency. Purified RNA should be stored at -70°C. If the anticipated RNA yield is over 30 µg, 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 (~13,400 × g) to get RNA.