Ver. 24081

ODP432

Plant Total RNA Kit

For extraction of total RNA from plant tissues

Plant Total RNA Kit

Spin column
(ODP432)

Kit Contents

Contents	50 Preps
Buffer RP	40 mL
Buffer RD	13 mL
Buffer RW	15 mL
RNase-free water	10 mL
Spin column CR3	50
Collection Tubes	50

Storage

Plant Total RNA kit can be stored dry at room temperature (15- 25°C) for up to 12 months without showing any reduction in performance and quality. For longer storage, the kit can be stored at 2-8°C.

(Note: Check buffers for precipitate before use and dissolve at 37°C for 10 minutes if necessary.)

Introduction

Plant Total RNA Kit provides a fast, simple, and cost- effective total RNA extraction method for routine molecular biology laboratory applications. The kit uses silica membrane technology to eliminate the cumbersome steps associated with loose resins or slurries. The kit is ready for use and can extract the total RNA from a wide variety of plant species and tissues, and the whole process is completed in less than 1 hour. Extracted RNA is ready-to-use in downstream applications such as: PCR, gene chips assay, northern blot, dot blot, poly A screening, in vitro transcript and molecular cloning.

Yield may vary relative to the type of sample, genomic size, ploidy, age of the sample etc. Young leaves or needles will give best result.

Chemicals required but not provided

1. Isopropanol
2. Chloroform
3. β-mercaptoethanol

Important Notes

1. Add appropriate amount of ethanol (96-100%) to Buffer RD and Buffer RW as indicated on the bottle before use.
   Note: It is advised to reconstitute ONLY required volume of Buffer RD with ethanol (96-100%) as reconstituted Buffer RD will precipitate on long term storage.
2. All centrifugation steps are carried out at 13,000 ×g (12,000 rpm) in conventional tabletop microcentrifuge at room temperature.
3. Increasing the time of absorption and elution could improve recovery efficiency of RNA.
4. The recovery efficiency is related to starting RNA quantity and elution volume. The less starting quantity or elution volume, less the recovery efficiency.

Protocol

Ensure that Buffer RD and Buffer RW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.

1. Place 100mg fresh sample or frozen sample in a 1.5mL microcentrifuge tube.
   Note: Samples may be frozen by dipping them in liquid nitrogen.
   Samples should be finely chopped before transferring to the microcentrifuge tube.
2. Add 700µL Buffer RP to the sample.
3. Add 20µL β-mercaptoethanol.
4. Vortex for 10 to 20 seconds to mix, make sure to disperse all clumps and incubate for 30 minutes at 65°C, vortex the tube for several times during incubation.
5. Add 700µL chloroform, mix by inverting the tube for several times, Centrifuge for 10 minutes at 12,000 rpm (~13,400 ×g).
6. Pipette the supernatant to a new tube, add 700µL ice-cold isopropanol, and mix by inverting the tube several times.
7. Pipette all of the mixtures from step 6, including any precipitate that may have formed, into the spin column (place the spin column in the collection tube). Close the lid, incubate for 10 minutes at room temperature and centrifuge for 30 seconds at 12,000 rpm (~13,400 ×g). Discard the filtrate and set the spin column into the collection tube.
   Note: Since the capacity of spin column CR3 is 700µL, the loading-centrifugation step must be repeated for processing all the mixture from step 6.
8. Carefully open the spin column and add 500µL Buffer RD (Ensure that ethanol (96-100%) is added to Buffer RD before use). Close the lid and centrifuge at 12000 rpm (~13,400 × g) for 30 seconds then discard the filtrate and place the spin column into the collection tube.
   Note: It is advised to reconstitute ONLY required volume of Buffer RD with ethanol (96-100%) as reconstituted Buffer RD will precipitate on long term storage.

9. Add 700µL RW (Ensure that ethanol is added to Buffer RW before use) to the spin column to wash the membrane, and centrifuge for 30 seconds at 12,000 rpm (~13,400 ×g), discard the flow-through, replace the spin column in the collection tube.
10. Repeat step 9.
11. Replace the spin column in the collection tube, centrifuge for 2 minutes at 12,000 rpm (~13,400 ×g) to remove residual wash Buffer RW. Discard the collection tube and transfer the spin column CR3 to a clean 1.5mL or 2mL microcentrifuge tube. Open the lid of the spin column CR3 and incubate the assembly at room temperature (15-25°C) 2 minutes to dry membrane completely.

    Note: Residual ethanol from Buffer RW may inhibit subsequent steps.
12. Pipette 50–200µL RNase-free water directly onto the membrane, incubate for 2-5 minutes at room temperature (15–25°C), and then centrifuge for 2 minutes at 12,000 rpm (~13,400 ×g) to elute.

    Note: The volume of RNase-free water must be more than 30µL, or it may affect recovery efficiency. To obtain higher productivity, add the solution gained from step 12 to the center of membrane again, let the column stand for 1 minute, and then centrifuge. Extracted RNA should be stored at –80°C.

Note:
For higher RNA yield the initial amount of sample can be increased. Buffer RP and β-mercaptoethanol may have to be added in higher quantity in such cases.