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Plant Total RNA Extraction Kit

Catalog No.	Specification	Storage/Shelf life
EP018	50T/200T	Room temperature/1 year

Introduction

The ground plant tissue is dissolved by the strong lysis solution and filtered to remove protein impurities and cell debris. Column purification technology efficiently removes residual protein and PCR inhibitors. After RNA is washed with solution RRPB and Solution RWB, it is eluted with RNase-Free ddH2O, which can be used in various molecular biology experiments.

Kit Components

Component	EP018-50T	EP018-200T
Lysis buffer C	**15 ml**	**60 ml**
RNA deproteinizing Solution RRPB	**30 ml*2**	**240 ml**
DNase I stock solution	**1 vial (stored at -20℃)**	**1 vial (stored at -20℃)**
DNase I Buffer RDB	**4ml**	**20 ml**
Wash Buffer RWB	**60 ml**	**240 ml**
Adsorption column R column	**50 sets**	**200 sets**
RNase-Free ddH2O	**40ml**	**160 ml**
User Manual	**1 copy**	**1 copy**

Before starting

RWB: Please add absolute ethanol to RWB (there is a label on the reagent bottle) before use. The **Lysis buffer C** may form a precipitate during storage. If there is a precipitate, it can be used after being dissolved in a 60-65°C water bath.

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Operation steps

1. Take 100 mg of plant sample and grind it with liquid nitrogen to powder, collect the powdered sample in a pre-cooled 1.5 mL EP tube, add 600 μl of **Lysis buffer C**, and add 2% β-mercaptoethanol (Can be added or not), fully shake Mix well.
   Note: For leaf samples, try to take new leaves or young leaf tips; for fruit, tuber, and petal samples, the recommended sample amount is 150 mg; for dried seeds such as red beans, the recommended sample amount is 60-100 mg, because Aspirate water and increase the **Lysis buffer C** to 1 ml volume.
2. Centrifuge at 12,000 rpm (~13,400 × g) for 5 min, and transfer the supernatant to a new 1.5mL EP tube for the following operations.
3. Slowly add 0.3 times the supernatant volume of isopropanol (provided by the user), and mix upside down (do not shake vigorously, precipitation may occur at this time), the resulting solution and precipitation are transferred to the **adsorption column R column** (adsorption Put the column in the collection tube), centrifuge at 10,000 rpm (~10,000 × g) for 1 min, discard the waste liquid in the collection tube, and put the **adsorption column R column** back into the collection tube. (The adsorption column can be loaded with 700μl solution at a time. If the solution and precipitation cannot be added at one time, please transfer to the **adsorption column R column** in multiple times).
4. Add 500 μl **Solution RRPB** to the R column of the adsorption column, and centrifuge at 10,000 rpm (~10,000 ×g) for 1 min.
5. Prepare DNase I working solution: Take 10 μl **DNase I stock solution** into a new RNase-Free EP tube, add 70 μl **DNase I buffer RDB**, and mix well (DNase I working solution is best prepared for current use).
6. Add 80 μl DNase I working solution to the R column of the adsorption column, and leave it at room temperature for 10 minutes.
7. Add 500 μl **Solution RRPB** to the R column of the adsorption column, and centrifuge at 10,000 rpm (~10,000 ×g) for 1 min.
8. Add 500 μl rinsing **wash buffer RWB** to the R column of the adsorption column (please check whether absolute ethanol has been added before use), let stand at room temperature for 2 min, centrifuge at 12,000 rpm (~13,400 ×g) for 1 min, discard the waste liquid, and Put the **adsorption column R column** back into the collection tube.
9. Repeat step 8.

10. Isolate centrifuge at 12,000 rpm (~13,400 × g) for 2 minutes and discard the waste liquid. Place the **adsorption column R column** in the ultra-clean table for a few minutes to thoroughly dry the remaining rinse liquid in the adsorption material.

    Note: The purpose of this step is to remove the residual rinsing liquid in the R column of the adsorption column. The residual rinsing liquid may affect subsequent RT and other experiments.

11. Transfer the adsorption column R to a new RNase-Free centrifuge tube, and drop 30-100 μl **RNase-Free ddH2O** into the middle of the adsorption membrane. Leave it at room temperature for 2 minutes at 12,000 rpm (~13,400 ×g) Centrifuge for 2 min to obtain RNA solution.

    Note: The volume of the elution buffer should not be less than 30 μl. Too small a volume will affect the recovery efficiency. Please store RNA solution at -70 ℃.

    RNA purity and concentration detection

    Integrity: RNA can be tested for integrity by ordinary agarose gel electrophoresis (electrophoresis conditions: gel concentration 1.2%; 1 ×TAE running buffer; 120V, 20 min). Since 70%-80% of RNA in the cell is rRNA, a very obvious rRNA band should be seen under UV after electrophoresis. The amount of 28S rRNA is about twice that of 18S rRNA, indicating that the integrity of the RNA is good.

    Purity: The ratio of OD_{260}/OD_{280} is a measure of the degree of protein contamination. For high-quality RNA, the OD_{260}/OD_{280} reading is between 1.8-2.1, and a ratio of 2.0 is a sign of high-quality RNA. The OD_{260}/OD_{280} reading is affected by the pH value of the solution used in the measurement. The same RNA sample, assuming that the OD_{260}/OD_{280} readings measured in a 10 mM Tris, pH 7.5 solution are between 1.8-2.1, the readings measured in an aqueous solution may be between 1.5-1.9, but this does not mean RNA Impure.

    Concentration: Take a certain amount of RNA extract, dilute it by n times with **RNase-Free ddH2O**, adjust the spectrophotometer to zero with **RNase-Free ddH2O**, take the diluted solution for OD_{260}/OD_{280} determination, and calculate the RNA concentration according to the following formula:

    Final concentration (ng/μl) = $(OD_{260})\times$(dilution factor n)×40