艾科瑞生物
Accurate Biology

Instruction for Use

SteadyPure Plant RNA Quick Extraction Kit

AG 21040
Version.V1E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This product allows rapid extraction of RNA from various plant tissues weighing less than 100 mg, including simple plants or complex plant parts such as roots, stems, leaves, flowers, fruits, and seeds. The kit features a unique lysis system that eliminates the need for toxic phenol-chloroform extraction. It rapidly lyzes tissues while inhibiting nucleases released from cells, preserving RNA integrity. Equipped with gDNA Eraser Mini Columns, it effectively removes impurities and genomic DNA. The Quick Plant RNA Mini Columns efficiently bind RNA, and the entire extraction process from sample homogenization takes only 10 minutes.

RNA obtained with this product is of high purity, with minimal contamination from proteins, genomic DNA, and other impurities. It can be directly used in RT-PCR, Real-Time RT-PCR, RNA library construction, Northern hybridization, in vitro translation, molecular cloning, chip analysis, and various other molecular biology experiments.

2. Kit Information

Kit Name	Cat. No	Specification
SteadyPure Plant RNA Quick Extraction Kit	AG 21040	50 rxns

3. Transportation and Storage

Storage	Store at Room Temperature
Transportation	Transport at Room Temperature

4. Kit Components

Buffer QPLS	30 ml
Buffer QWA	30 ml
Buffer QWB*1	30 ml
RNase Free Water*2	10 ml
gDNA Eraser Mini Columns	50 Sets
Quick Plant RNA Mini Columns	50 Sets
Collection tubes	50 pcs
RNase Free tubes*3	50 pcs

*1. Before first use, add 70 ml of anhydrous ethanol to Buffer QWB (Buffer QWB to anhydrous ethanol volume ratio 3:7). Mix thoroughly, label the bottle, and store at room temperature.

*2. RNase-Free Water should be stored at -20°C after opening.

*3. This component is solely for RNA elution. Centrifuge tubes used during the initial lysis step need to be prepared separately.

5. Not Provided Experimental Materials

Anhydrous Ethanol, 1.5 mL RNase Free Centrifuge Tube.

6. Recommendation and Experimental Precautions

1) Use fresh biological materials whenever possible to ensure the extracted RNA is not degraded.
2) When grinding tissue with liquid nitrogen, add liquid nitrogen continuously to prevent RNA degradation.
3) Typically, the starting amount of plant tissue should be between 20 mg and 100 mg. Do not exceed the maximum starting amount, and ensure thorough lysis to avoid clogging the Mini Column, which can affect RNA yield and purity.
4) The gDNA Eraser Mini Columns in this product effectively remove genomic DNA. For complete digestion of gDNA, you may choose our product【DNase I (RNase Free) Code No. AG12001】.
5) The maximum loading volume for the Quick Plant RNA Mini Column is 750 µL. If the sample exceeds this volume, use multiple columns for purification or load the sample in multiple steps: load 750 µL, centrifuge, discard the filtrate, and then load the remaining mixture, repeating the process.
6) During operation, carefully remove the adsorption column vertically from the collection tube or 1.5 ml centrifuge tube (or place it back), avoiding contact with the tube wall to prevent contamination.
7) To prevent RNase contamination during operation, pay attention to the following:

a) Use a dedicated RNA workbench, frequently change gloves, wear RNA-certified lab coats, minimize talking and movement during operation, and avoid passing objects over the centrifuge tubes.

b) Strictly prevent RNase contamination from the environment, containers, consumables, and reagents used.

7. Protocol

7.1 Lysis Step

1) Transfer an appropriate amount of fresh or -80°C frozen plant tissue sample into a liquid nitrogen-chilled mortar. Grind the tissue thoroughly with a pestle (continuously replenish liquid nitrogen during grinding) until a fine powder is obtained (no visible particles; insufficient grinding may reduce yield).

Note: The typical starting amount of plant tissue is 20 mg~100 mg; adjust based on experimental results.

Note: Alternatively, a bead mill homogenizer may be used. Transfer the sample into a grinding tube containing 600 µL Buffer QPLS, then homogenize following the manufacturer's instructions. Proceed directly to <Lysis Step 3> after homogenization.

2) Transfer the ground powder into a 1.5 ml RNase-free tube containing 600 µL Buffer QPLS, then vortex vigorously for 1 min to ensure complete lysis.

Note: For difficult-to-lyse plant tissues, extend vortexing up to 5 min to improve RNA yield.

3) Centrifuge at 12,000 rpm for 2 min (room temperature), then proceed immediately to the purification steps.

7.2 Purification Step

1) Carefully transfer the supernatant to the gDNA Eraser Mini Column and centrifuge at 12,000 rpm for 1 min (room temperature).

Note: The pellet contains plant debris. Avoid transferring the pellet to ensure RNA purity.

Note: Maximize RNA yield by aspirating as much supernatant as possible without disturbing the pellet.

2) Discard the gDNA Eraser Mini Column. Precisely measure the filtrate volume and transfer it to a new 1.5 ml RNase-free tube.
3) Add 0.5x filtrate volume of absolute ethanol to the filtrate. Mix thoroughly by pipetting. If viscous or precipitated material is observed, pipette repeatedly to disperse.
4) Immediately transfer the mixture to the Quick Plant RNA Mini Column and centrifuge at 12,000 rpm for 30 sec (room temperature). Discard the flow-through.
5) Add 500 µl Buffer QWA to the Quick Plant RNA Mini Column and centrifuge at 12,000 rpm for 30 sec (room temperature). Discard the flow-through.
6) Add 700 µl Buffer QWB to the Quick Plant RNA Mini Column and centrifuge at 12,000 rpm for 30 sec (room temperature). Discard the flow-through.

Note: Ensure Buffer QWB has been supplemented with the specified volume of absolute ethanol.

7) Optional Step (Please refer to section: **gDNA Digestion**).
   If gDNA removal is required, proceed with the <Optional Step>.
   If not required, proceed directly to <Purification Step 8>.
8) Add another 700 µl Buffer QWB to the Quick Plant RNA Mini Column and centrifuge at 12,000 rpm for 30 sec (room temperature). Discard the flow-through.
9) Place the Quick Plant RNA Mini Column into a new 2.0 ml Collection Tube and centrifuge at 12,000 rpm for 2 min (room temperature).
10) Place the Quick Plant RNA Mini Column onto a new RNase-Free Tube. Add 50–200 µl RNase-Free Water directly to the center of the column membrane, then centrifuge at 12,000 rpm for 2 min (room temperature) to elute the RNA. The resulting RNA solution can be used immediately for downstream applications or stored at -80°C.

Note: Pre-warming RNase-Free Water to 50–65°C before use improves elution efficiency.

For higher RNA yield, the eluate from <Purification Step 10> can be reloaded onto the same Quick Plant RNA Mini Column for a second elution.

Note: The volume of RNase-Free Water can be adjusted based on desired RNA concentration:

For higher concentrations, reduce volume (e.g., 30–50 µl).

For standard applications, use 50–200 µl.

Optional Step: DNase I Digestion

1) Prepare the DNase I Reaction Mix according to the table below and mix thoroughly. Add 50 µL of the reaction mix directly to the center of the Quick Plant RNA Mini Column membrane. Incubate at room temperature for 15 minutes.

Components	Volume
DNase I(RNase free)	4 µL
10X DNase I Buffer	5 µL
RNase Free Water	41 µL

2) Add 350 µL Buffer QWB to the center of the Quick Plant RNA Mini Column membrane. Centrifuge at 12,000 rpm for 30 sec (room temperature) and discard the flow-through.
3) Continue with Purification Steps 8-10 as described above.

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