艾科瑞生物
Accurate Biology

Instruction for Use

SteadyPure Plant RNA Extraction Kit

AG 21019
Version.V1E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This Product is optimized for extraction of total RNA from plant tissues or fungal input of 50 ~ 100 mg, especially those rich in polysaccharides, polyphenols, or starch (roots, stems, leaves, flowers, fruits, seeds), with extraction protocols provided for common plant tissues. The kit is based on silica gel membrane purification technology without time-consuming alcohol precipitation and does not require phenol/chloroform, or any other toxic reagent during the extraction process.

This kit is designated with two different workflows, for normal plant samples and plant samples with high polysaccharides/polyphenols/starch abundance. The retrieved RNA product is with high purity and low contamination of protein, genomic DNA and other impurities, and could be used for downstream experiments including RT-PCR, Real Time RT-PCR, cDNA library construction, Northern Blot, Dot Blot, Poly(A) screening, in vitro translation, RNase protection analysis, molecular cloning, bio-chip analysis.

2. Kit Information

Kit Name	Cat. No	Specification
SteadyPure Plant RNA Extraction Kit	AG 21019	50 rxns

3. Transportation and Storage

	Package 2-1	Package 2-2
Storage	Store at –20°C	Store at Room Temperature
Transportation	Transport at –20°C Dry Ice or Blue Ice Condition	Transport at Room Temperature

4. Kit Components

Package 2-1 (-20 °C Storage)

Kit Components	Volume
50 x DTT Solution	700 µl
DNase I (RNase Free)	200 µl
10 x DNase I Buffer	1 ml

Package 2-2 (Room Temperature Storage)

Kit Components	
Buffer PPS	25 ml
Buffer PA-2	1.5 ml x 2
Buffer RLS	1.5 ml x 2
Buffer RWA	30 ml
Buffer RWB*1	30 ml
RNase free water*2	13 ml
Plant RNA Mini Columns	50 sets
Collection Tubes	50 pcs
RNase Free Tubes*3	50 pcs

*1: Before use, add 70 ml of 100% ethanol (Ratio of Buffer RWB:100% ethanol is 3:7) to the Buffer RWB bottle. Mix well and properly mark the bottom. Keep at room temperature for storage.

*2: Recommend to store at -20 °C after the first use.

*3: Only use for final elution step. Prepare tubes for lysis steps.

5. Not Provided Experimental Materials

100% Ethanol, 1.5 ml centrifuge tubes (RNase Free), 2.0 ml centrifuge tubes (RNase Free).

6. Recommendation and Experimental Precautions

(1) For precipitant in Buffer PPS, heat and dissolve at 37 °C. For precipitant in Buffer RLS, heat and dissolve at 60 °C. Then retain to room temperature before use.

(2) For Buffer RWB, add 70 ml of 100% Ethanol to the bottle before use. Mix well and mark properly.

(3) For Buffer RLS, add 20μL of 50 x DTT solution for each 1 ml of Buffer RLS (making a final concentration of 1 x DTT solution).
*This shall be conducted right before experiment. Solution added with 50 x DTT solution should not be stored more than 1 month.

(4) Recommend to use freshly collected samples to reduce RNA degradation.

(5) Constantly add liquid nitrogen to keep cool operating temperature for sample grinding.

(6) Do not add more than 700 μL each time of solution into the Plant RNA Mini Column, otherwise would affect the efficiency and purity, even cause failure of extraction.

(7) Do not add more than recommended 100 mg upper limit of input samples, otherwise would affect the efficiency and purity.

(8) Avoid touching the tube wall when adding or relocating solutions. Do not touch the membrane of the tube.

(9) Recommend to adopt the DNase I Digestion step in this protocol, to eliminate genomic DNA/gDNA contamination.

(10) Adopt adequate protection procedures and gears. Recommend to operate in RNA processing designated lab area to reduce RNA degradation and environmental contamination.

(11) Must use RNase Free consumables.

7. Protocol

7.1 Sample Lysis Treatment

For normal sample

1. Grind the plant sample (fresh or frozen at -80 °C) into powder in liquid nitrogen, using a mortar. Grind it into small particle as possible, while keep adding liquid nitrogen to keep operation in cool temperature.
2. Retrieve 50 ~ 100 mg of grind powder to a tube with 500 µL of Buffer RLS (Buffer RLS added with 50 x DTT Solution). Vortex thoroughly to lyse well.
3. Settle at room temperature for 2 min, then centrifuge at 4°C, 12,000 rpm for 5 min.
4. Retrieve and relocate the supernatant to a new 1.5 ml centrifuge tube (RNase Free).

For sample with high polysaccharides/polyphenols/starch

1. Grind the plant sample (fresh or frozen at -80 °C) into powder in liquid nitrogen, using a mortar. Grind it into small particle as possible, while keep adding liquid nitrogen to keep operation in cool temperature.
2. Retrieve 50 ~ 100 mg of grind powder to a tube with 500 µL of Buffer PPS. Vortex thoroughly to lyse well.
3. Settle at room temperature for 2 min, then centrifuge at 4°C, 12,000 rpm for 5 min. Relocate the supernatant to a new 1.5 ml centrifuge tube (RNase Free). Add PA-2 (of 1/10 volume of the supernantant volume) to supernatant, vortex and mix for about 15 sec. Then centrifuge again at 4°C, 12,000 rpm for 5 min.
4. Retrieve and relocate the supernatant to a new 2.0 ml centrifuge tube (RNase Free). Add 500 µL of Buffer RLS (Buffer RLS added with 50 x DTT Solution) again, Vortex thoroughly to lyse well until no visible precipitant.

7.2 Extraction/Purification

1. Add 100% ethanol (of 1/2 volume of the lysis solution) to the lysis solution retrieved from step 7.1. Use pipette to mix well until no visible precipitant. (Precipitant will affect the recover rate and purity of purification)
2. Relocate all lysis solution from above to the Plant RNA Mini Column, centrifuge at room temperature, 12,000 rpm for 2 min. Discard the filtrate in the bottom tube, retrieve the tube. (If the lysis solution is more than 700 µL, add no more than 700 µL each time and repeat until all lysis filtered)
3. Add 600 µL of Buffer RWA into the Plant RNA Mini Column, centrifuge at room temperature, 12,000 rpm for 1 min .Discard the filtrate in the bottom tube, retrieve the tube.
4. Add 750 µL of Buffer RWB (Buffer RWB with 100% ethanol added) into the Plant RNA Mini Column, centrifuge at room temperature, 12,000 rpm for 1 min .Discard the filtrate in the bottom tube, retrieve the tube.
5. **(Optional)** DNase I digestion **(Option when the sample is expected with high genomic DNA contamination or leftovers)**
   (1) Prepare DNase I Digestion mix as below. Mix well.

Components	Volume
DNase I (RNase Free)	4 µL
10 x DNase I Buffer	5 µL
RNase free water	41 µL

   (2) Add 50 µL of Digestion Mix onto center of the membrane of Plant RNA Mini Column. Settle at room temperature for 15 min.
   (3) Add 350 µL of Buffer RWB onto the membrane, centrifuge at room temperature, 12,000 rpm for 1 min .Discard the filtrate in the bottom tube, retrieve the tube.
6. Add 750 µL of Buffer RWB onto Plant RNA Mini Column, centrifuge at room temperature, 12,000 rpm for 1 min.Discard the filtrate in the bottom tube, retrieve the tube.
7. Relocate the top tube of the Plant RNA Mini Column onto a new 2.0 ml Collection tube. Centrifuge at room temperature, 12,000 rpm for 2 min.
8. Relocate the top tube of the Plant RNA Mini Column onto a new RNase Free centrifuge tube. Add 50 ~ 200 µL of RNase Free Water, settle at room temperature for 5 min, then centrifuge at room temperature, 12,000 rpm for 2 min. The retrieved solution in the bottom centrifuge tube is the RNA product elution.Store the RNA product elution at -80 °C, or intermediately run Reverse Transcription.

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