艾科瑞生物
Accurate Biology

Instruction for Use

SteadyPure Quick RNA Extraction Kit

AG 21023
Version.V1E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This product enables the rapid extraction of total RNA from ≤1.0E+07 cultured cells (such as HL60, 293T) and ≤10 mg of tissue samples (such as heart, liver, kidney, brain, stomach). The kit utilizes a unique lysis system, eliminating the need for phenol-chloroform extraction. The system quickly lyses cells or tissues while inhibiting nuclease activity, thereby preserving RNA integrity. Advantages of the kit include simplicity, speed, high purity, and high yield. The purified total RNA is suitable for use in various molecular biology experiments, including RT-qPCR, RT-PCR, Northern blotting, mRNA purification, and in vitro translation.his product.

2. Kit Information

Kit Name	Cat. No	Specification
SteadyPure Quick RNA Extraction Kit	AG 21023	50 rxns

3. Transportation and Storage

Storage	Store at Room Temperature
Transportation	Transport at Room Temperature

4. Kit Components

Kit Components	Volume
Buffer QLS	30 ml
Buffer QWB*1	15 ml
RNase Free Water*2	10 ml
Quick RNA Mini Columns	50 sets
Collection Tubes	50 pcs
RNase Free Tubes*3	50 pcs

*1: Before first use, add 35 ml of 100% ethanol to Buffer QWB (volume ratio of Buffer QWB to anhydrous ethanol is 3:7). Mix thoroughly and label the bottle. Store at room temperature.

*2: It is recommended to store this component at -20°C after opening.

*3: This component is intended for RNA elution only; centrifuge tubes used for initial lysis need to be provided separately.

5. Not Provided Experimental Materials

(1) Materials required but not provided: anhydrous ethanol, PBS buffer, 1.5 ml centrifuge tubes (RNase free).

(2) If Buffer QLS shows precipitation, heat at 50-60°C to dissolve it. Allow the solution to return to room temperature before use.

(3) Before first use, add 35 ml of 100% ethanol to Buffer QWB (volume ratio of Buffer QWB to anhydrous ethanol is 3:7). Mix thoroughly and label the bottle. Store at room temperature.

6. Recommendation and Experimental Precautions

(1) Fresh experimental materials should be used to ensure that the extracted RNA is not degraded.

(2) When grinding tissue samples with liquid nitrogen, add liquid nitrogen continuously to ensure that the RNA is not degraded; if using a tissue grinder, ensure low-temperature grinding and thorough homogenization.

(3) The recommended sample input amount in this manual is suitable for most samples. For samples with extremely high or low nucleic acid content, adjust the sample starting amount as needed.

(4) RNA extraction must be performed at room temperature and should not be placed on ice until RNA is obtained after elution centrifugation. This prevents the formation of insolubles that could clog the RNA purification column.

(5) Ensure samples are fully lysed to prevent clogging of the Mini Column, which can affect RNA yield and purity. For larger samples, increase the reagent volume and use multiple Mini Columns for purification.

(6) The maximum volume of the Quick RNA Mini Column is 700 µL. If the liquid volume exceeds this capacity, add in batches.

(7) Prevent RNase contamination during the operation by following these guidelines:

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

b) Prevent RNase contamination in the operating environment, containers, consumables, and reagents used.Please adopt adequate lab management and experimental procedures to control RNase contamination.

7. Protocol

7.1 Lysis

For different sample types, select the appropriate lysis steps. If sample size increases or nucleic acid content is excessively high, proportionally increase the lysis buffer volume and use multiple Mini Columns for purification.

Lysis of Suspension Cells:

(1) Centrifuge ≤1.0E + 07 suspension cells at 8,000 x g and 4°C for 2 minutes, collecting the cells at the bottom of the centrifuge tube. Discard the supernatant.

(2) Wash the collected cells with 1x PBS buffer, then centrifuge at 8,000 x g and 4°C for 2 minutes. Discard the supernatant.

(3) Add 500 µL of Buffer QLS lysis solution to the centrifuge tube containing the cells.

(4) Immediately mix thoroughly using high-speed vortexing or by repeatedly pipetting until the lysate is clear and non-viscous.

(5) Settle the lysate at room temperature for 2 minutes.

Lysis of Adherent Cells:

(1) Aspirate the culture medium from the culture dish and wash the cells once with 1x PBS buffer.

(2) Remove the PBS buffer, then add 500 µL of Buffer QLS lysis solution to ≤1.0E + 07 adherent cells. Gently shake the culture dish to ensure the Buffer QLS solution is evenly distributed over the cell surface.

Note: For firmly adherent cells, use a cell scraper to detach them and proceed immediately with the following steps.

(3) Use a pipette to repeatedly pipette the solution to detach the cells. Transfer the entire volume of the lysate containing the cells to a centrifuge tube. Mix thoroughly using high-speed vortexing or by repeated pipetting until the lysate is clear and non-viscous.

(4) Settle the lysate at room temperature for 2 minutes.

Lysis of Tissue Samples:

A. Liquid Nitrogen Grinding

(1) Transfer an appropriate amount of fresh or -80°C frozen tissue sample to a pre-chilled mortar. Grind the tissue sample with a pestle, adding liquid nitrogen continuously during the grinding process, until a fine powder is achieved (no visible particles; insufficient grinding may affect RNA yield).

(2) Transfer ≤10 mg of the powdered tissue sample to a 1.5 ml RNase-free centrifuge tube containing 300 µL of Buffer QLS lysis solution. Mix thoroughly by high-speed vortexing or by repeated pipetting until the sample is fully lysed. Any residual unlysed precipitate can be removed in subsequent centrifugation steps.

(3) Centrifuge at 12,000 rpm, 4°C , for 5 minutes.

(4) Carefully transfer the supernatant to a new 1.5 ml RNase-free centrifuge tube.

B. Grinding with a Pestle or Tissue Homogenizer

(1) Transfer ≤10 mg of fresh or -80°C frozen tissue sample to a 1.5 ml RNase-free centrifuge tube or grinding tube containing 300 µL of Buffer QLS lysis solution. Grind the sample thoroughly using a pestle or tissue homogenizer until fully lysed. If the homogenate volume is less than 300 µL after grinding, add additional Buffer QLS lysis solution to make up to 300 µL, then vortex to mix well. Any residual unlysed precipitate can be removed in subsequent centrifugation steps.

(2) Centrifuge at 12,000 rpm, 4°C, for 5 minutes.

(3) Carefully transfer the supernatant to a new 1.5 ml RNase-free centrifuge tube.

7.2 Purification

1. Add an equal volume of 100% ethanol to the above homogenate, and mix thoroughly by pipetting. If noticeable viscosity or precipitation occurs, pipette several times to disperse the precipitate.

(**Note:** Failure to disperse the precipitate may cause blockage of the Quick RNA Mini Column, affecting yield and purity.)

2. Immediately transfer the entire mixture to the Quick RNA Mini Column, centrifuge at 12,000 rpm at room temperature for 2 minutes, and discard the filtrate.

3. If DNA digestion is required, proceed with the optional steps below. If not, continue with the following steps.

4. Add 700 µL of Buffer QWB to the Quick RNA Mini Column, centrifuge at 12,000 rpm at room temperature for 1 minute, and discard the filtrate.

(**Note:** Ensure that the specified volume of 100% ethanol has been added to Buffer QWB.)

5. Place the Quick RNA Mini Column onto a new 2.0 ml Collection Tube and centrifuge at 12,000 rpm at room temperature for 2 minutes.

(**Note:** Remove the column vertically to prevent the column tip from touching the collection tube wall. Placing it in a new 2.0 ml Collection Tube helps improve RNA purity.)

6. Transfer the Quick RNA Mini Column to a new RNase-Free Tube, add 50 µL-200 µL of RNase-Free Water to the center of the column membrane, let it sit at room temperature for 3 minutes, then centrifuge at 12,000 rpm at room temperature for 2 minutes to elute the RNA. Store the eluted RNA at -80°C.

Optional Step: DNase I Digestion, using our DNase I (RNase-Free) product :

1. Prepare the DNase I reaction mixture as per the table below and mix well. Add 50 µL of DNase I reaction mixture to the center of the Quick RNA Mini Column membrane, and incubate at room temperature for 15 minutes.

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

2. For subsequent steps, follow the purification steps 4-6 as outlined above.

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