艾科瑞生物
Accurate Biology

Instruction for Use

AG *RNAex Pro* Reagent

AG 21101
Version.V3E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This Product is optimized to extractt Total RNA from animal tissues, plant materials, various microorganisms, cultured cells, and other sources. Samples are thoroughly lysed in RNAex, and after the addition of chloroform and centrifugation, the solution will separate into an upper aqueous phase, an interphase, and an organic phase (the bright red lower layer containing proteins, polysaccharides, fatty acids, cellular debris, and DNA). RNA is distributed in the upper aqueous phase. The upper aqueous phase should be collected carefully, ensuring that the interphase is not collected. Total RNA can then be recovered through isopropanol precipitation.

Using RNAex to extract Total RNA can be completed within 1 hour. The extracted Total RNA is of high purity, containing minimal proteins and genomic DNA, and can be directly used for various molecular biology experiments such as Northern blotting, mRNA purification, in vitro translation, and RT-PCR. If necessary, DNase I treatment can be further employed to remove any trace amounts of DNA from the Total RNA.

2. Kit Information and Component

Kit Name	Cat. No	Specification
AG *RNAex Pro* Reagent	AG 21101	100 ml

3. Transportation and Storage

Storage	Store at 4 °C, Avoid Light Exposure
Transportation	Transport at Room Temperature

4. Not Provided Experimental Materials

Chloroform, Iso propanol, 80% Ethanol (pre-cooled at -20 °C), RNase-Free Water

5. Recommendation and Experimental Precautions

(1) The utensils used in the experiment should be carefully protected from RNase contamination.

(2) During the experiment, disposable gloves and masks should be worn for all reagent preparation and experimental procedures to avoid RNase contamination.

(3) Strong denaturants may be encountered during the experiment. Appropriate lab attire, goggles, masks, and gloves should be worn before beginning any experimental procedures.

Note: Used solutions must be collected in waste containers for specialized disposal. If a spill occurs, rinse immediately with plenty of water, followed by cleaning with a 1% sodium hypochlorite solution. For detailed information, refer to the relevant MSDS.

6. Protocol

Sample Type	Sample Input	RNAex Input Volume
Tissue Sample	50-100 mg	1 ml
Adherent Cell	1 x 10^6-2 x 10^7	1 ml
Suspension Cell	1 x 10^6-2 x 10^7	1 ml

6.1 Lysis

Adherent Cells

(1) The culture medium should be aspirated, and the cells should be washed once with 1 x PBS.

(2) The PBS wash should be aspirated, and then 1 ml of RNAex should be added to each 1 x 10^6 - 2 x 10^7 cultured cells. The dish should be gently shaken to ensure the RNAex solution is evenly distributed over the cell surface.

(Note: For strongly adherent cells, a cell scraper can be used to detach the cells.)

(3) A pipette should be used to repeatedly pipet the solution to detach the cells. The lysate containing the cells should then be transferred to a centrifuge tube and pipetted until no visible precipitate remains (the solution should be clear).

(4) After standing at room temperature for 5 minutes, proceed with subsequent RNA extraction steps.

Suspension Cells

(1) Suspension cells shall be collected in a centrifuge tube and centrifuged at 8,000 g, 4°C for 2 minutes, discarding the supernatant.

(2) Add 1 ml of RNAex to each 1 x 10^6 - 2 x 10^7 cells.

(3) A pipette should be used to repeatedly pipet until the lysate is clear with no visible precipitate.

(4) After standing at room temperature for 5 minutes, proceed with subsequent RNA extraction steps.

Animal Tissue and Plant Material Samples

(1) The accurately weighed RNA extraction sample should be transferred to a pre-cooled mortar with liquid nitrogen and ground with a pestle until it becomes a powder (continuously add liquid nitrogen during grinding). Then, an appropriate amount of RNAex should be added to the powder and mixed well. (For soft and easily lysed tissue samples, a homogenizer with an appropriate amount of RNAex can be used for homogenization and lysis.)

(2) The mixture should be transferred to a centrifuge tube and pipetted repeatedly to mix thoroughly. After standing at room temperature for 5 minutes, centrifuge at 12,000 g, 4°C for 5 minutes.

(3) Carefully aspirate the supernatant and transfer it to a new centrifuge tube, then proceed with subsequent RNA extraction steps.

6.2 Extraction

(1) Add chloroform equivalent to 1/5 of volume of RNAex to the lysate, and mix thoroughly. Settle at room temperature for 5 minutes.

(2) Centrifuge at 12,000 g, 4°C for 15 minutes. Carefully remove the centrifuge tube. The homogenate will separate into three layers: the upper aqueous phase (containing RNA), the interphase (protein layer), and the lower organic phase.

(3) Carefully transfer the upper aqueous phase to a new centrifuge tube, avoiding the interphase.

(4) Add isopropanol equivalent to 1/2 the volume of RNAex to the aqueous phase, and mix thoroughly. Settle at room temperature for 10 minutes.

(5) Centrifuge at 12,000 g, 4°C for 10 minutes. Discard the supernatant carefully, ensuring not to disturb the RNA pellet.

(6) Add 80% ethanol (pre-cooled at -20°C) equivalent to the volume of RNAex to the centrifuge tube to wash the RNA pellet and the tube walls. Centrifuge at 7,500 g, 4°C for 5 minutes. Carefully discard the supernatant without touching the pellet.

(**Note:** When discarding the supernatant, remove as much as possible. A pipette can be used to aspirate residual droplets from the tube walls and mouth.)

(7) Open the centrifuge tube cap and air-dry the pellet at room temperature or under vacuum for about 5 minutes.

(**Note:** For significant ethanol residue, extend the drying time. Do not use centrifugation or heating to dry, which will make the RNA difficult to dissolve.)

(8) Add an appropriate amount of RNase-free water to the centrifuge tube to dissolve the RNA. Store the dissolved RNA at -80°C.

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