艾科瑞生物
Accurate Biology

Instruction for Use

SteadyPure RNA Extraction Kit (with RNAex Pro Lysis)

AG 21024
Version.V1E2

Research Use Only
Not For Diagnosis Procedures

1. Description

This product is an RNA extraction kit without lysis buffer, containing purification columns and washing buffers. It is designed to be used in conjunction with AG RNAex Pro Reagent. By combining the RNAex and column membrane purification methods, it enables the rapid extraction of total RNA from samples such as ≤2.0E+07 cultured cells and ≤100 mg of tissue. After sample lysis with RNAex, the mixture can be combined directly with ethanol without chloroform extraction (the chloroform-free method), or chloroform extraction can be performed, followed by mixing the supernatant with ethanol (the chloroform extraction method). The lysate mixed with ethanol is then transferred to the RNA Mini Column for further RNA purification. Impurities and salts are removed using washing buffers, and high-purity RNA is finally eluted with RNase Free Water. The purified total RNA can be directly used for various molecular biology experiments such as RT-qPCR, RT-PCR, Northern blotting, mRNA purification, and next-generation sequencing.

If other lysis buffers are to be used with this product, it is recommended to conduct preliminary studies to confirm the extraction efficacy. Additionally, the appropriate sample starting amount and lysis buffer volume should be determined based on the different lysis buffers used.

2. Kit Information

Kit Name	Cat. No	Specification
SteadyPure RNA Extraction Kit (with RNAex Pro Lysis)	AG 21024	50 rxns

3. Transportation and Storage

Storage	Store at Room Temperature
Transportation	Transport at Room Temperature

4. Kit Components

Kit Components	Volume
Buffer RW1	30 ml
Buffer RW2*1	27 ml
RNase Free Water*2	13 ml
RNA Mini Columns	50 sets
Collection Tubes	50 pcs
RNase Free Tubes*3	50 pcs

*1: Before the first use of Buffer RW2, add 63 ml of 100% ethanol (Buffer RW2 to absolute ethanol volume ratio is 3:7). Mix well and label the bottle. Store at room temperature.

*2: After opening, it is recommended to store at -20°C.

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

5. Not Provided Experimental Materials

Required materials (not included): Absolute ethanol, 70% ethanol, PBS, chloroform (optional), 1.5 ml centrifuge tubes (RNase free).

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 materials with liquid nitrogen, liquid nitrogen should be added continuously to ensure that the extracted RNA is not degraded.

(3) The sample amount should not exceed the maximum starting amount to avoid insufficient sample lysis or clogging of the Mini Column, which can affect RNA yield and purity. If the sample starting amount is large, the amount of lysis reagent (such as RNAex) can be appropriately increased to ensure sufficient sample lysis.

(4) The maximum volume of the RNA Mini Column is 700 μL. If the volume of the liquid exceeds the maximum capacity during use, it can be added in batches: load 700 μL of the lysate, centrifuge, discard the filtrate, then load the remaining lysate and repeat this step.

(5) To prevent RNase contamination during the operation, attention should be paid to the following aspects:

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

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

c) Use nuclease-free water to accurately prepare the 70% ethanol solution, and ensure that the prepared 70% ethanol is free from nuclease contamination.

7. Recommendations for Sample Input and Lysis Volume

The adequate lysis of the sample is crucial for obtaining the desired yield and purity. If the amount of lysis buffer is fixed, an excessive sample amount can lead to insufficient lysis, clogging of the purification column, and ultimately, reduced RNA yield and purity. For example, with AG RNAex Pro Reagent, the lysis buffer usage and sample starting amount can be referenced below. If the sample amount is too large, please increase the amount of RNAex used.

Sample Type	Chloroform-Free Extraction		Chloroform Extraction	
	Sample Input	RNAex Lysis Volume*1	Sample Input	RNAex Lysis Volume*1
Adherent cells	≤ 1X 10^7	0.6 ml	≤ 2X 10^7	1 ml
Suspension cells	≤ 1X 10^7	0.6 ml	≤ 2X 10^7	1 ml
Animal tissue	≤ 30 mg	0.6 ml	≤ 50 mg	1 ml
Plant tissue	≤ 60 mg	0.6 ml	≤ 100 mg	1 ml
Yeast cells	≤ 3X 10^7	0.6 ml	≤ 5X 10^7	1 ml
Bacteria	≤ 2X 10^9	0.6 ml	≤ 4X 10^9	1 ml
Blood*2	/	/	0.25 ml	Up to 1 ml

*1: Experimentation according to the recommended RNAex amounts in this table requires centrifugation on the column twice. If the sample starting amount is less than the recommended amount in the table, to ensure thorough lysis, it is advised not to alter the amount of RNAex used but to add according to the recommended amounts in the table. If the sample volume increases, it is recommended to proportionally increase the amount of RNAex used and perform multiple column centrifugations.

*2: For blood samples, the chloroform extraction method is recommended. If the sample starting volume is less than 0.25 ml, add RNAex to reach a final volume of 1 ml. If the sample starting volume is greater than 0.25 ml, add RNAex in an amount three times the sample starting volume.

8. Protocol

8.1 Lysis Procedure

This procedure is exemplified using AG RNAex Pro Reagent. The addition amounts of the lysis buffer and the samples can be referenced in the above table.

Adherent Cells

1.Remove the culture medium and wash the cells once with 1x PBS.
2.Remove the PBS wash, then add an appropriate amount of RNAex to the adherent cells. Gently shake the culture dish to ensure uniform distribution of the RNAex solution on the cell surface. **(Note: For firmly adherent cells, use a cell scraper to detach the cells.)**
3.Use a pipette to repeatedly pipette up and down to dislodge the cells. Then transfer the lysate containing cells to a centrifuge tube and pipette up and down until there is no visible precipitate in the lysate (clear solution).
4.Let it stand at room temperature for 5 minutes before proceeding to the subsequent RNA purification steps.

Suspension Cells

1. Collect an appropriate amount of suspension cells into a centrifuge tube, centrifuge at 8,000 g for 2 minutes at room temperature, and discard the supernatant.
2. Add an appropriate amount of RNAex to the cell pellet.
3. Pipette up and down until the lysate is clear without any visible precipitate.
4. Let it stand at room temperature for 5 minutes before proceeding to the subsequent RNA purification steps.

Animal Tissues, Plant Tissues, Yeast Samples

1. Add accurately weighed samples to a pre-chilled mortar containing liquid nitrogen and grind the tissue with a pestle (during grinding, continually add liquid nitrogen to the mortar). Grind until it becomes a powder, then add an appropriate amount of RNAex and mix well. (For soft and easily lysed tissue samples, a homogenizer can also be used to add an appropriate amount of RNAex for homogenization and lysis.)
2. Transfer the mixture to a centrifuge tube and pipette up and down to mix thoroughly. Let it stand at room temperature for 5 minutes, then centrifuge at 12,000 g for 5 minutes at 4°C.
3. Carefully transfer the supernatant to a new centrifuge tube and proceed with the subsequent RNA purification steps.

Bacteria

1. Collect an appropriate amount of bacteria into a centrifuge tube, centrifuge at 12,000 rpm for 2 minutes at room temperature, and discard the supernatant.
2. Lysozyme digestion step: This step is optional. If higher yields are required, perform this step according to "Optional Step 1" in the appendix. If not needed, proceed with step 3 below. (RNA can be extracted from Gram-negative bacteria without lysozyme, but higher yields can be obtained with lysozyme; it is recommended to perform lysozyme digestion for Gram-positive bacteria.)
3. Add an appropriate amount of RNAex and pipette up and down until the bacterial cells are completely lysed.
4. Let it stand at room temperature for 5 minutes before proceeding to the subsequent RNA purification steps.

Blood Samples (Using Chloroform Extraction)

1.Transfer fresh or frozen blood to a centrifuge tube and quickly add RNAex to the blood (adjust the blood volume according to the table.
2.Pipette up and down repeatedly until the cells are completely lysed. Let it stand at room temperature for 5 minutes before proceeding to the subsequent RNA purification steps.

8.2 Purification Procedure

Choose one of the following purification methods (both methods effectively remove genomic DNA and proteins. If higher requirements for gDNA removal are needed, DNase I digestion can be performed in subsequent steps):

Step 1

Method 1: Chloroform-Free Extraction:

Add an equal volume of 70% ethanol to the lysate obtained in the previous step, mix thoroughly by pipetting. If there is obvious viscous material or precipitate, pipette multiple times to disperse the precipitate, then proceed to purification step 2.

Method 2: Chloroform Extraction:

(a) Add 200 µL of chloroform (1/5 of the volume of the lysate) to the lysate, mix thoroughly. Let it stand at room temperature for 5 minutes.

[The volume of the lysate refers to the total volume after adding the sample. For blood samples, the lysate volume refers to the total volume of blood and RNAex.]

(b) Centrifuge at 12,000 g for 15 minutes at 4°C. Carefully remove the centrifuge tube. The homogenate is separated into three layers: supernatant (containing RNA), middle protein layer, and lower organic phase.

(c) Carefully transfer the supernatant to another new centrifuge tube (do not aspirate the middle protein layer). Add an equal volume of 70% ethanol to the supernatant, mix thoroughly by pipetting. If there is obvious viscous material or precipitate, pipette multiple times to disperse the precipitate. Proceed to purification step 2.

Step 2-8

2. Immediately transfer all of the above mixture to the RNA Mini Column. Centrifuge at 12,000 rpm for 1 minute at room temperature, discard the filtrate.
3. Add 600 µL of Buffer RW1 to the RNA Mini Column. Centrifuge at 12,000 rpm for 1 minute at room temperature, discard the filtrate.
4. Add 650 µL of Buffer RW2 to the RNA Mini Column. Centrifuge at 12,000 rpm for 1 minute at room temperature, discard the filtrate. (Note:Ensure that Buffer RW2 contains the specified volume of 100% ethanol)
5. DNA digestion step: This step is optional. If higher requirements for gDNA removal are needed, perform this step according to **"Optional Step 2"** in the appendix. If not needed, proceed with step 6 below.
6. Add 650 µL of Buffer RW2 to the RNA Mini Column. Centrifuge at 12,000 rpm for 1 minute at room temperature, discard the filtrate.
7. Place the RNA Mini Column on a new 2.0 ml Collection Tube, centrifuge at 12,000 rpm for 2 minutes at room temperature. **(Note: Remove the adsorption column vertically to avoid the column head touching the wall of the collection tube; installing it on a new 2.0 ml Collection Tube helps to improve RNA purity).**
8. Place the RNA Mini Column on a new RNase-free tube, add 50 µL~200 µL of RNase-free Water to the center of the membrane of the RNA Mini Column.Settle at room temperature for 5 minutes. Centrifuge at 12,000 rpm for 2 minute to elute the RNA. The dissolved RNA shall be directly used or stored at -80°C.

Appendix

Optional Step 1: Lysozyme Digestion

1. Add an appropriate amount of lysozyme to the bacterial pellet and mix well by pipetting to fully suspend the bacteria. (It is recommended to add 100 µl of 0.5 mg/ml lysozyme to ≤ 2 X 10^9 bacterial samples. If the amount of bacteria increases, adjust the volume of lysozyme proportionally.)
2. Heat at 37°C for 10 minutes (extend heating time to 30 minutes for bacteria that are difficult to lyse).
3. Centrifuge at 12,000 rpm for 2 minutes at room temperature, discard the supernatant.
4. Follow the subsequent experimental steps as described in steps 3-4 of the bacterial lysis procedure.

Optional Step 2: DNase I Digestion [DNase I (RNase Free) (Code. AG12001) from AG or equivalent]

1. Prepare the DNase I reaction mixture according to the table below and mix well.

Components	Use Volume
DNase I (RNase Free)	4 µl
10 x DNase I Buffer	5 µl
RNase Free Water*2	41 µl

2. Add 50 µl of the DNase I reaction mixture to the center of the membrane of the RNA Mini Column. Let it stand at room temperature for 15 minutes.
3. Add 350 µl of Buffer RW2 to the center of the membrane of the RNA Mini Column. Centrifuge at 12,000 rpm for 1 minute at room temperature, discard the filtrate.
4. Follow the subsequent RNA purification steps as described in steps 6-8 above.

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