ELK Biotechnology

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FFPE RNA Extraction Kit

Catalog No.	Specification	Storage/Shelf life
EP031	50T	Room temperature/1 year

Storage Conditions: DNase I and 10×Reaction Buffer are stored at -20°C, other components can be stored at room temperature (15-30°C)

Kit Components

Component	EP031 50 preps
DNase I	1000 U
10×Reaction Buffer	1000 µL
Buffer DS	30 mL
Buffer GTL	15 mL
Buffer GL	25 mL
Proteinase K	12.5 mg
Proteinase K Storage Buffer	1.25 mL
Buffer RW1	40 mL
Buffer RW2（concentrate）	11 mL
RNase-Free Water	10 mL
Spin Columns DM with Collection Tubes	50
RNase-Free Centrifuge Tubes (1.5 mL)	50

Introduction

This kit is suitable for effectively purifying total RNA from formalin-fixed and paraffin-embedded tissues. It is suitable for extracting high-purity total RNA from wax-embedded tissues or sections weighing less than 30 mg. This kit does not require the use of phenol/chloroform extraction and isopropanol precipitation. Multiple samples can be

extracted within one hour. This product uses specially optimized lysis buffer and proteinase K to release RNA from formalin-fixed or tissue section samples. No overnight operation is required; after digestion of the sample, incubation at a higher temperature removes the inhibitory effect caused by formalin cross-linking, effectively releasing RNA from tissue sections while avoiding damage to the integrity of RNA; the optimized buffer system enables RNA in the lysis buffer to specifically bind to the silica gel adsorption membrane, while other contaminants can flow through the membrane; it can be effectively removed through the rinsing step, and the eluted RNA can be directly used for RT-PCR, Real-Time PCR and blotting analysis and other experiments.

Reagents to Be Supplied by User

100% ethanol, 10 mM PBS (PH7.4)

Precautions

1. Add 0.625 mL Proteinase K Storage Buffer to Proteinase K to dissolve it and store at -20°C. Do not store the prepared Proteinase K at room temperature for a long time, and avoid repeated freezing and thawing, so as not to affect its activity.
2. To prevent RNase contamination, the following aspects should be noted:

2.1 Use RNase-free plastic products and pipette tips to avoid cross-contamination.

2.2 Glassware should be dry-baked at 180℃ for 4 hours before use, plasticware can be soaked in 0.5 M NaOH for 10 minutes, rinsed thoroughly with water and then autoclaved.

2.3 RNase-free water should be used for the preparation of the solution.

2.4 Operators wear disposable masks and gloves, and gloves should be changed frequently during the experiment.

3. After obtaining the sample, fix the sample in 4%-10% formalin as soon as possible. The fixation time is preferably 14-24 h. If the time is too long, the RNA will be broken and the downstream experiments will be affected. Make sure the sample is thoroughly dehydrated before embedding, residual formalin will inhibit the action of Proteinase K.

4. Before the first use, add 100% ethanol to Buffer RW2 according to the instructions on the reagent bottle label.

5. Please check Buffer GTL, Buffer GL and Buffer DS for crystals or precipitation before use.

If there is crystal or precipitation, please re-dissolve Buffer GTL, Buffer GL and Buffer DS in a 56°C water bath.

Protocol

1. Sample processing

1.1 1a. Paraffin-embedded samples: Use a scalpel to trim off excess paraffin from the tissue block to expose the tissue and cut into 5-10 µm slices.

Note: If the sample surface has been exposed to air, discard the 2-3 pieces exposed to air.

1.2 1b. Samples in a fixative such as formalin: Take about 20 mg of the sample, cut into small pieces, put in a centrifuge tube, add 500 µL of 10 mM PBS (pH 7.4), vortex, 12,000 rpm (~13,400 ×g) Centrifuge for 1 minute, discard the supernatant, repeat 3 times, and proceed directly to step 3.

2. Choose plan A or plan B to remove paraffin

Scenario A

A1. Take about 1×1 cm2 slices (about 4-5 slices in total) and put them in a centrifuge tube (self-provided), add 500 µl Buffer DS, and vortex for 10 seconds. Incubate at 56°C for 3 minutes.

A2. Centrifuge at 12,000 rpm for 2 minutes, carefully aspirate the supernatant, taking care not to aspirate the pellet.

Option B

B1. Take a slice of about 1×1 cm^2 (about 4-5 slices in total) and place it in a centrifuge tube (self-provided), add 1 ml of xylene, close the tube cap tightly, and vortex for 10 seconds.

B2. Centrifuge at 12,000 rpm for 2 minutes, carefully aspirate the supernatant, taking care not to aspirate the pellet.

B3. Add 1 mL 100% ethanol, vortex to mix. Centrifuge at 12,000 rpm for 2 minutes, discard the supernatant, taking care not to aspirate the pellet.

B4. Open the cap and incubate at room temperature or up to 37°C for 10 minutes until no ethanol remains.

3. Add 150 µL Buffer GTL to resuspend the pellet; add 10 µl Proteinase K, and mix by vortexing.

4. Incubate at 56°C for 15 minutes until the sample is completely dissolved. Incubate at 80°C for 15 minutes. Briefly centrifuge to collect the solution on the tube wall to the bottom of the tube.

Note: 1) The purpose of this step is to repair the nucleic acid denatured by formaldehyde. High temperature or long incubation time may cause RNA fragmentation and RNA fragmentation.

2) The samples incubated at 56°C can be placed at room temperature until the temperature of the water bath or dry bath reaches 80°C, and then the samples are incubated at 80°C.

5. Place on ice for 3 minutes, centrifuge at 12,000 rpm for 15 minutes, transfer the supernatant to a new centrifuge tube, being careful not to aspirate the pellet.
6. Add 320 µL Buffer GL to the supernatant, and vortex to mix thoroughly.
7. Add 720 µL 100% ethanol and vortex to mix thoroughly.

Note: After adding 100% ethanol, a small amount of precipitate may be precipitated, but it will not affect subsequent operations.

8. Add all the solution obtained in step 7 to the adsorption column (Spin Columns RS) that has been loaded into the collection tube. If the solution cannot be added at one time, it can be transferred in multiple times. Centrifuge at 12,000 rpm for 1 minute, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

Optional step: To remove genomic DNA, follow the steps below.

8.1 Add 350 µL of Buffer RW1 to the adsorption column, centrifuge at 12,000 rpm for 1 minute, discard the waste liquid, and put the adsorption column back into the collection tube.

8.2 To prepare DNase I mix: Take 52 µL RNase-Free Water, add 8 µL to it Mix 10×Reaction Buffer and 20 µL DNase I (1 U/µL) to prepare a reaction solution with a final volume of 80 µL.

8.3 Add 80 µL DNase I mixture directly to the adsorption column and incubate at 20-30°C for 15 minutes.

8.4 Add 350 µL of Buffer RW1 to the adsorption column, centrifuge at 12,000 rpm for 1 minute, discard the waste liquid, and put the adsorption column back into the collection tube.

9. Add 500 µL Buffer RW2 to the adsorption column (check whether 100% ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
10. Repeat step 9.
11. Centrifuge at 12,000 rpm for 2 minutes and discard the waste in the collection tube. Allow the cartridge to dry at room temperature for several minutes.

Note: The purpose of this step is to remove residual ethanol in the adsorption column, which will affect subsequent enzymatic reactions (enzymatic digestion, PCR, etc.).

12. Place the adsorption column in a new RNase-free centrifuge tube, add 20-50 µL RNase-Free Water to the middle of the adsorption column, stand at room temperature for 2-5 minutes, centrifuge at 12,000 rpm for 1 minute, collect the RNA solution, Store RNA at -20°C.

Note: 1) The volume of RNase-Free Water should not be less than 20 µL, and the recovery

rate will be affected if the volume is too small.

2) To increase the yield of RNA, repeat step 12 with 20-50 μL of new RNase-Free Water.

3) If you want to increase the RNA concentration, you can re-add the obtained solution to the adsorption column and repeat step 12.