Fixed Tissue RNA Extraction Kit

Project number: R669871

Storage conditions: -20° C.

Products

individual parts making up a compound	50T
DNase I	1000U
10×Reaction Buffer	1000μl
Buffer DS	30ml
Buffer GTL	15ml
Buffer GL	25ml
Proteinase K	12.5mg
Proteinase K Storage Buffer	1.25ml
Buffer RW1	40ml
Buffer RW2 (concentrate)	11ml
RNase-Free Water	10ml
Spin Columns RS with Collection Tubes	50
RNase-Free Centrifuge Tubes (1.5 ml)	50

Products

This kit is suitable for the efficient purification of total RNA from formalin-fixed, paraffin-embedded tissues. Suitable for extracting total RNA of higher purity from less than 30 mg of paraffin-embedded tissues or sections. This kit eliminates the need for phenol/chloroform extraction and isopropanol precipitation, and allows for the extraction of multiple samples in less than an hour. This kit is designed for the efficient purification of total RNA from formalin-fixed, paraffin-embedded tissues or sections. This product uses specially optimized lysate and proteinase K to release RNA from formalin-fixed or tissue section samples without overnight manipulation; digested samples are incubated at higher temperatures to remove inhibition caused by formalin cross-linking, effectively releasing RNA from tissue

sections without jeopardizing the integrity of the RNA; the optimized buffer system allows the RNA in the lysate to bind specifically to the silica gel The optimized buffer system allows the RNA in the lysate to specifically bind to the silica gel adsorbent membrane, while other contaminants can flow through the membrane; it can be effectively removed by the rinsing step, and the eluted RNA can be directly used in RT-PCR, Real-Time PCR, and blotting analysis experiments.

Self-contained reagents: anhydrous ethanol (freshly opened or for RNA extraction), 10 mM PBS (PH7.4).

Pre-experiment Preparation and Important Notes

1. Add 0.625ml Proteinase K Storage Buffer to Proteinase K to dissolve it and store it at -20℃. Do not leave the prepared Proteinase K at room temperature for a long time and avoid repeated freezing and thawing to avoid affecting its activity.
2. To prevent RNase contamination, attention should be paid to the following aspects:
1) Use RNase-free plastics and tips to avoid cross-contamination.
(2) Glassware should be dry-roasted at 180° C for 4 hours before use, and plasticware can be soaked in 0.5M NaOH for 10 minutes, rinsed thoroughly with water and autoclaved.
3) RNase-free water should be used to prepare the solution.
(4) Operators wear disposable masks and gloves, and change gloves diligently during the experiment.
3. After obtaining the samples, fix the samples in 4%-10% formalin as soon as possible, and the fixation time should be 14-24 hours; too long a period of time will easily lead to RNA breakage and affect the downstream experiments.
4. Ensure that the sample is thoroughly dehydrated before embedding; residual formalin will inhibit Proteinase K.
5. Anhydrous ethanol should be added to Buffer RW2 according to the label instructions of the reagent bottle before first use.
Check Buffer GTL, Buffer GL, and Buffer DS for crystallization or precipitation before use, and if crystallization or precipitation occurs, redissolve Buffer GTL, Buffer GL, and Buffer DS in a 56° C water bath.

Procedure

1. Sample processing
1a. Paraffin-embedded samples: Trim excess paraffin from the tissue block with a scalpel to expose the tissue and cut into 5-10 μm slices.
Note: If the surface of the sample has been exposed to air, discard the 2-3 pieces that were exposed to air and do not use them.
1b. Sample in fixative such as formalin: take about 20 mg of sample, cut into small pieces, place in centrifuge tube, add 500 μl of 10 mM PBS (PH7.4), vortex shaking, centrifuge for 1 minute at 12,000 rpm (~ 13,400×g), discard the supernatant, and repeat 3 times, which can be used directly to proceed to step 3.
2. Select Option A or Option B Paraffin Removal

Scenario A

A1. Take about 1 x 1 cm2 sections (about 4-5 sections in total) in a centrifuge tube (supplied), add 500 μl Buffer DS and vortex and shake for 10 s. Incubate at 56° C for 3 min.

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

Option B

B1. Take about 1×1 cm2 slices (about 4-5 slices in total) and place them in a centrifuge tube (prepared by yourself), add 1 ml of xylene, cover the tube tightly and vortex for 10 seconds.

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

B3. Add 1 ml of anhydrous ethanol and mix by vortexing and shaking. Centrifuge at 12,000 rpm for 2 min and discard the supernatant, taking care not to aspirate the precipitate.

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

3. Add 150 μl Buffer GTL, resuspend the precipitate; add 10 μl Proteinase K, vortex and shake to mix.

4. incubate at 56° C for 15 minutes until the sample is completely dissolved. incubate at 80° C for 15 minutes. Centrifuge briefly so that the solution on the wall of the tube collects at the bottom of the tube.

Note: 1) The purpose of this step is to repair the nucleic acid denatured by formaldehyde, the incubation temperature is too high or too long may cause RNA breakage and produce RNA fragments.

(2) Samples after incubation at 56° C may be left at room temperature until the water or dry bath temperature reaches 80° C before the samples are placed at 80° C for incubation.

5. Place on ice for 3 min, centrifuge at 12,000 rpm for 15 min, and transfer the supernatant to a new centrifuge tube, being careful not to aspirate the precipitate.

6. Add 320 μl Buffer GL to the supernatant and mix thoroughly with vortex shaking.

7. Add 720 μl of anhydrous ethanol and mix thoroughly with vortex shaking.

Note: A small amount of precipitate may precipitate after the addition of anhydrous ethanol, but it does not affect the subsequent operation.

8. Add all of the solution from step 7 to the Spin Columns RS in the collection tube, or in several passes if the solution cannot be added all at once. centrifuge at 12,000 rpm for 1 minute, pour off the waste from the collection tube, and return the column to the collection tube.

Optional step: If genomic DNA is to be removed, the following steps can be followed

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

b. Preparation of DNase I mixture: Take 52 μl of RNase-Free Water, add 8 μl of 10×Reaction Buffer and 20 μl of DNase I (1U/μl) to it, mix well, and make a final volume of 80 μl of reaction solution.

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

d. Add 350 μl of Buffer RW1 to the adsorption column, centrifuge at 12,000 rpm for 1 min, discard the waste solution, and put the adsorption column back into the collection tube.

9. Add 500 μl Buffer RW2 to the adsorption column (check that anhydrous ethanol has been added before use), centrifuge the column at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and put the column back into the collection tube.

10. Repeat step 9.

11. Centrifuge at 12,000 rpm for 2 minutes and pour off the waste liquid in the collection tube. Leave the adsorption column at room temperature for several minutes to dry thoroughly.

Note: The purpose of this step is to remove residual ethanol from the adsorption column; ethanol residue can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).

12. Place the adsorbent column in a new RNase-free centrifuge tube and add 20-50 μl to the center of the adsorbent column overhang.

RNase-Free Water, leave at room temperature for 2-5 minutes, centrifuge at 12,000 rpm for 1 minute, collect the RNA solution, and store the RNA at -20 ° C.

Note: 1) The volume of RNase-Free Water should not be less than 20 μl, too small a volume affects the recovery rate. 2) If you want to increase the yield of RNA, repeat step 12 with 20-50 μl of fresh RNase-Free Water.

3) If the RNA concentration is to be increased, the resulting solution can be reintroduced into the adsorption column and step 12 repeated.