FFPE DNA/RNA Kit

Fixed Tissue DNA/RNA Extraction Kit

Catalog number: F666120 (50 preps)

Storage conditions: DNase I and 10×Reaction Buffer -20℃, Spin Columns DF and Spin Columns RS can be stored at room temperature for 2 months, 2-8℃ for 1 year, the rest of the components are stored at room temperature (15-30℃).

Products Content:

Component	50 preps
DNase I	1000 U
10×Reaction Buffer	1000 μl
RNase A (100 mg/ml)	0.4 ml
Buffer GTL	20 ml
Buffer GL	30 ml
Buffer GW1 (concentrate)	13 ml
Buffer GW2 (concentrate)	15 ml
Buffer RW1	40 ml
Buffer RW2 (concentrate)	11 ml
Buffer EB	10 ml
RNase-Free Water	10 ml
Buffer DS	30 ml
Proteinase K	2 x 25 mg
Proteinase K Storage Buffer	2 x 1.25 ml
Spin Columns RS with Collection Tubes	50
Spin Columns DF with Collection Tubes	50
Centrifuge Tubes (L-1.5 ml)	100

Products Introduction

This kit is suitable for the effective purification of genomic DNA and total RNA from paraffin-embedded tissues, using specially optimized deparaffinizing agents and lysates to release DNA and RNA from tissue section samples, without the use of the organic reagent xylene, and without the need for overnight operation; the digested samples are incubated at higher temperatures to remove inhibitors caused by cross-linking, which can effectively improve nucleic acid yields and purity; and an optimized buffer system allows nucleic acids in the lysate to bind

specifically to the adsorbent membrane, and inhibitors are effectively removed by a two-step rinsing procedure. The optimized buffer system enables the nucleic acids in the lysate to bind specifically to the adsorbent membrane, and the inhibitors are effectively removed by a two-step rinsing step, and finally eluted with low-salt buffer or water to obtain high purity DNA and RNA, and at the same time, equipped with a high-efficiency microsorbent column, the volume of the elution can be as low as 20 μl. The purified DNA and RNA can be directly used for PCR, Real-time PCR, SNP genotyping, STR genotyping, and so on. The purified DNA and RNA can be directly used for PCR, Real-time PCR, SNP genotyping, STR genotyping, second-generation sequencing, pharmacogenomics research and blot analysis.

Self-contained reagent: anhydrous ethanol

Pre-experiment Preparation and Important Notes

1. After obtaining the sample, fix the sample as soon as possible, the fixation time of 14-24 hours is appropriate, too long a period of time will easily lead to

DNA and RNA breaks, affecting downstream experiments. If the formaldehyde fixation time is too long or the sample is stored for too long

(>1 year) is prone to compromise DNA integrity and failure to amplify long fragments.

2. Ensure that samples are thoroughly dehydrated prior to embedding; residual formalin will inhibit Proteinase K action.

3. Add 1.25 ml of Proteinase K Storage Buffer to Proteinase K to dissolve it, and store at -20℃. Do not leave the prepared Proteinase K at room temperature for a long period of time to avoid affecting its activity.

4. Anhydrous ethanol should be added to Buffer RW2, Buffer GW1 and Buffer GW2 according to the label instructions on the vials before first use.

5. Check Buffer GTL, Buffer GL and Buffer DS for crystallization or precipitation prior to use; if crystallization or precipitation occurs, redissolve Buffer GTL, Buffer GL and Buffer DS in a 37° C water bath.

6. Preheat the water bath or thermostatic mixer to 56° C before starting the experiment.

7. Use an ambient temperature centrifuge or set the centrifuge temperature to 25° C. Temperatures below 15° C may result in clogging of the adsorption column.

8. To prevent RNase contamination, the following should be observed:

1) Use RNase-free plastics and tips to avoid cross-contamination.

(2) Glassware should be dry baked at 180° C for 4 hours before use, plasticware can be soaked in 0.5 M 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.

procedure

Paraffin-embedded samples

1. Trim off excess paraffin from the tissue block to expose the tissue and cut into 5-10 μm slices.

2. Place approximately 1 x 1 cm2 slices (1-5 slices in total) in a centrifuge tube (supplied), add 500 μl of Buffer DS and vortex for 10 s. Briefly centrifuge the sample to the bottom of the tube. Centrifuge briefly to collect the sample at the bottom of the tube, incubate at 56° C for 3 minutes, remove from the water bath and allow to cool to room temperature before proceeding.

Note: If the surface of the sample is exposed to air, discard the initial 2-3 slices without using them.

3. Centrifuge at 12,000 rpm for 2 minutes and carefully discard the supernatant thoroughly without aspirating the precipitate. The residual dewaxing solution can be carefully removed with a small tip (10 μl).

4. Add 180 μl of Buffer GTL and 20 μl of Proteinase K to the above tube and mix well with vortexing.

5. Incubate at 56° C for 15 minutes, then place on ice for 3 minutes. Centrifuge at 12,000 rpm for 15 minutes at room temperature.

6. Transfer the supernatant to a new 1.5 ml centrifuge tube for RNA extraction, taking care not to aspirate undigested tissue. Use the precipitate for DNA extraction.

RNA extraction

7. Take the supernatant obtained in step 6 and incubate at 80° C for 15 minutes.

8. Add 320 μl of Buffer GL, mix by vortexing and shaking, then add 720 μl of anhydrous ethanol and mix immediately by vortexing and shaking.

9. Add all of the resulting solution to the Spin Columns RS in the collection tube; if the solution cannot be added all at once, it may be transferred in several passes. centrifuge the column at 12,000 rpm for 1 minute, pour off the waste solution from the collection tube, and place the column back into the collection tube. **Note: If the columns are clogged, the sample size may be too large and consideration should be given to reducing the number of starting sections to 1-2.**

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

a. Add 350 μl of Buffer RW1 to the column, centrifuge at 12,000 rpm for 1 minute, discard the waste solution, and place the column back into the collection

tube.

b. Preparation of DNase I mixture: Take 52 μl of RNase-Free Water and add 8 μl of 10×Reaction to it.

Buffer and 20 μl DNase I (1 U/μl), mix well, and prepare a final volume of 80 μl of reaction solution.

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

d. Add 350 μl of Buffer RW1 to the column, centrifuge at 12,000 rpm for 1 minute, discard the waste solution, and return the column to the collection tube.

Add 500 μl of Buffer RW2 to the adsorbent column, centrifuge at 12,000 rpm for 1 min, pour off the waste liquid in the collection tube, and put the adsorbent column back into the collection tube.

11. Repeat step 10. centrifuge at 12,000 rpm for 2 minutes and pour off the waste liquid in the collection tube. Place the column at room temperature for 5 minutes.

minutes to dry thoroughly.

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

RNase-Free Water, left at room temperature for 5 minutes, centrifuged at 12,000 rpm for 1 minute, and collected RNA solution, the

-80° C for storage.

DNA extraction

7. Take the precipitate obtained in step 6 and add 180 μl Buffer GTL and 20 μl Proteinase K to the precipitate. Vortex

Resuspend the precipitate for 15 seconds.

8. Incubate at 56° C for 1 hour until the sample is completely dissolved. 90° C for 1 hour.

Add 200 μl Buffer GL, vortex and shake to mix and then add 200 μl anhydrous ethanol, vortex and shake to mix thoroughly. Centrifuge briefly so that the solution on the wall of the tube collects at the bottom of the tube.

Add all of the solution from step 9 to the Spin Columns DF in the collection tube, or transfer the solution in several passes. centrifuge at 12,000 rpm for 1 minute, pour off the waste solution from the collection tube, and return the column to the 10. collection tube.

Note: If the adsorption column is clogged, the sample size may be too large and consideration should be given to reducing the number of starting sections to 1-2.

11. Add 500 μl of Buffer GW1 to the adsorbent column and centrifuge at 12,000 rpm for 1 minute. Pour off the waste liquid from the collection tube and put the column back into the collection tube.

12. Add 500 μl of Buffer GW2 to the adsorbent column and centrifuge at 12,000 rpm for 1 minute. Pour off the waste liquid from the collection tube and place the column back into the collection tube.

Note: Step 12 may be repeated if further purity is required.

13. 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 5 minutes to dry thoroughly.

Note: The purpose of this step is to remove residual ethanol from the adsorbent column; ethanol residue will affect the subsequent enzymatic reaction.

14. Place the column in a new 1.5 ml centrifuge tube, add 20-50 μl Buffer EB to the center of the column, leave at room temperature for 5 minutes, centrifuge at 12,000 rpm for 1 minute, collect the DNA solution, and store at -20℃.