11. Repeat step 10. Centrifuge at 12,000 rpm for 2 minutes and discard the waste in the collection tube. Allow the adsorption column to dry at room temperature for 5 minutes. Place the adsorption column in a new RNase-free centrifuge tube, add 20-50 μL RNase-Free
12. Water to the middle of the adsorption column, stand at room temperature for 5 minutes, centrifuge at 12,000 rpm for 1 minute, collect the RNA solution, store RNA at -80°C.

DNA extraction

7. Add 180 μL Buffer GTL and 20 μL Proteinase K to the pellet obtained in step 6. Vortex for 15 seconds to resuspend the pellet.
8. Incubate at 56°C for 1 hour until the sample is completely dissolved. Incubate at 90°C for 1 hour.

Optional step: To remove RNA, lower the temperature of the sample to room temperature, add 2 μL of RNase A (100 mg/mL), shake and mix well, and leave at room temperature for 2 minutes.

9. Add 200 μL of Buffer GL, vortex to mix, then add 200 μL of absolute ethanol, and vortex to mix thoroughly. Briefly centrifuge to collect the solution on the tube wall to the bottom of the tube.
10. Add all the solution obtained in step 9 to the adsorption column (Spin Columns DF) that has been loaded into the collection tube, centrifuge at 12000 rpm for 1 minute, pour out the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
    Note: If the adsorption column is blocked, there may be too many samples, so we should consider reducing the number of initial slices to 1-2.
11. Add 500 μL of Buffer GW1 to the adsorption column (please check whether 100% ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
12. Add 500 μL of Buffer GW2 to the adsorption column (please 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.
    Note: To further improve DNA purity, repeat step 12.
13. Centrifuge at 12,000 rpm for 2 minutes, and discard the waste liquid in the collection tube. Allow the adsorption column to dry at room temperature for 5 minutes.
    Note: The purpose of this step is to remove the residual ethanol in the adsorption column, which will affect the subsequent enzymatic reaction.
14. Place the adsorption column in a new 1.5 mL collection tube, add 20-50 μL of Buffer TE or sterilized water to the middle of the adsorption column, stand at room temperature for 5 minutes, centrifuge at 12,000 rpm for 1 minute, and collect the DNA solution, stored at -20°C.

Version: 01/2024

FFPE DNA/RNA Kit

Cat. No. : CW3145S (50 preps)

Storage Conditions:

DNase I and 10×Reaction Buffer store at -20°C. Spin Columns DF and Spin Columns RS could be stored at room temperature for 2 months, at 2-8°C for 1 year. And the other components are stored at room temperature (15-30°C).

Components

Component	CW3145S 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	2×25 mg
Proteinase K Storage Buffer	2×1.25 mL
Spin Columns RS with Collection Tubes	50
Spin Columns DF with Collection Tubes	50
Centrifuge Tubes (L-1.5 mL)	100

Introduction

The kit is suitable for efficiently purification of genomic DNA and total RNA from paraffin-embedded tissues. This product uses specially optimized dewaxing agent and lysis solution to release DNA and RNA from tissue slice; it does not involve xylene and does not need to operate overnight. After the digested sample is incubated at a higher temperature, the inhibition effect caused by crosslinking is removed, which effectively improves the yield and purity of DNA. The optimized buffer system enables the DNA in the lysate to be specifically bound to the adsorption membrane. High-purity DNA can be obtained by effectively removing inhibitors through a two-step rinsing step, followed by final elution with low-salt buffer or water. At the same time, it is equipped with a high-efficiency micro-adsorption column, and the elution volume can be as low as 20 μL. The purified DNA can be used directly for PCR, Real-time PCR, SNP genotyping, STR genotyping, next-generation sequencing pharmacogenomics research and imprinting analysis.

Reagents to Be Supplied by User: 100% ethanol

Important Points Before Starting

1. After obtaining the sample, fix the sample as soon as possible, and the fixation time should be 14-24 hours. If the time is too long, the genome will be broken and the downstream experiments will be affected. If the formaldehyde fixation time is too long or the sample is stored for a long time (>1 year), the integrity of the DNA is easily damaged, and long fragments cannot be amplified.
2. Make sure that the sample before embedding is completely dehydrated, and the residual formalin will inhibit the effect of Proteinase K.
3. Add 1.25 mL Proteinase K Storage Buffer to Proteinase K to dissolve it, and store at -20°C. Prolonged storage of formulated Proteinase K at room temperature will affect its activity.
4. Before the first use, add absolute ethanol to Buffer RW2、Buffer GW1 and Buffer GW2 according to the instructions on the reagent bottle label.
5. Before use, please check whether Buffer GTL, Buffer GL and Buffer DS are crystallized or precipitated. If there is crystallisation or precipitation, please re-dissolve Buffer GTL, Buffer GL and Buffer DS in a water bath at 37°C.
6. Before starting the experiment, preheat the water bath or constant temperature mixer to 56°C.
7. Please use a centrifuge at room temperature, or set the centrifuge temperature to 25°C. If the temperature is lower than 15°C, the adsorption column may be blocked.
8. To prevent RNase contamination, attention should be paid to the following aspects.
   1) Use RNase-free plastics and gun tips to avoid cross-contamination.
   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.
   3) RNase-free water should be used for the preparation of the solution.
   4) Operators should wear disposable masks and gloves, and change gloves regularly during the experiment.

Procedure

1. Trim off excess paraffin from the tissue block to expose the tissue and cut into 5-10 μm slices.
2. Take about 1×1 cm^2 slices (about 1-5 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. After taking it out of the water bath, cool it to room temperature and proceed to the next operation.
   Note: If the sample surface is exposed to air, discard the initial 2-3 slices.
3. Centrifuge at 12,000 rpm for 2 minutes, carefully aspirate the supernatant, taking care not to aspirate the pellet. Residual dewaxing solution can be carefully removed with a small gun tip (10 μL).
4. Add 180μL of Buffer GTL and 20μL of Proteinase K into the above tube, and mix by vortexing.
5. Incubate at 56°C for 15 minutes, then place on ice for 3 minutes, centrifuge at 12,000 rpm for 15 minutes.
6. Transfer the supernatant to a new centrifuge tube for RNA extraction, being careful not to aspirate the pellet. Use the pellet for DNA extraction.

RNA extraction

7. Incubate the supernatant obtained in step 6, at 80°C for 15 minutes.
8. Add 320 μL Buffer GL to the supernatant, and vortex to mix thoroughly. Add 720 μL absolute ethanol and vortex to mix thoroughly.
9. Add all the solution obtained 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.
   Note: If the adsorption column is blocked, there may be too many samples, so we should consider reducing the number of initial slices to 1-2.

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

a. 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.
b. To prepare DNase I mix: Take 52 μL RNase-Free Water, add 8 μL 10×Reaction Buffer and 20 μL DNase I (1 U/μL) to prepare a reaction solution with a final volume of 80 μL.
c. Add 80 μL 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 minute, discard the waste liquid, and put the adsorption column back into the collection tube.

10. Add 500 μL Buffer RW2 to the adsorption column, 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.