5. Add 200 μL of Buffer GL, vortex to mix, then add 200 μL of 100% ethanol, and vortex to mix thoroughly. Briefly centrifuge to collect the solution on the tube wall to the bottom of the tube.
   Note: 1) Mix well immediately after adding Buffer GL and 100% ethanol.
   2) After adding Buffer GL and 100% ethanol, a white precipitate may occur, which will not affect subsequent experiments.
   3) If you need to operate on multiple samples, you can mix Buffer GL and 100% ethanol before adding the samples.
6. Add all the solution obtained in step 5 to the adsorption column (Spin Columns DF) that has been loaded into the collection tube, centrifuge at 25°C, 12000 rpm for 2 minutes, pour out the waste liquid in the collection tube, and put the adsorption column back into the collection tube. .
7. Add 500 μL of Buffer GW1 to the adsorption column (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.
8. Add 500 μL of Buffer GW2 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.
   Note: To further improve DNA purity, repeat step 8.
9. Centrifuge at 12,000 rpm for 2 minutes, and discard the waste liquid in the collection tube. Allow the cartridge to dry at room temperature for several minutes.
   Note: The purpose of this step is to remove the residual ethanol in the adsorption column, which will affect the subsequent enzymatic reaction.
10. Place the adsorption column in a new 1.5 mL collection tube, add 20-100 μL of Buffer TE or sterilized water to the middle of the adsorption column, stand at room temperature for 2-5 minutes, centrifuge at 12,000 rpm for 1 minute, and collect the DNA solution , stored at -20°C.
    Note: 1) The pH value of the eluent has a great influence on the elution efficiency. If water is used as the eluent, the pH value should be ensured at 7.0-8.5. When the pH value is lower than 7.0, the elution efficiency is not high.
    2) If you want to increase the final concentration of DNA, you can re-add the DNA eluate obtained in step 10 to the adsorption membrane, place at room temperature for 2 minutes, and centrifuge at 12,000 rpm for 1 minute.

Version: 12/2023

NuClean FFPE DNA kit

Cat. No. : CW2646S (50 preps)

Storage Condition: The Spin Columns DF can be stored at room temperaturefor two month and 2-8°C for up to one year. Other components can be stored at room temperature (15-30°C) .

Components

Component	CW2646S 50 preps
RNase A	0.4 mL
Buffer GTL	15 mL
Buffer GL	15 mL
Buffer GW1 (concentrate)	13 mL
Buffer GW2 (concentrate)	15 mL
Buffer TE	10 mL
Buffer DS	30 mL
Proteinase K	2×25 mg
Proteinase K Storage Buffer	2×1.25 mL
Spin Columns DF with Collection Tubes	50
Centrifuge Tubes (1.5 mL)	50

Introduction

This kit is suitable for purification of genomic DNA from formalin-fixed, paraffin-embedded tissues(FFPE). The kit uses optimized deparaffinization reagents and lysis buffers to release DNA from formalin-fixed or tissue sectioned samples, is xylene-free, and requires no overnight manipulation. After the digested sample is incubated at a higher temperature, it is cross-linked with formalin that removes free DNA, 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 and pharmacogenomics research.

The molecular weight of DNA isolated from formalin-fixed, paraffin-embedded samples is generally lower than that from fresh or frozen samples. The degree of DNA fragmentation depends on the sample type, storage time, and fixation conditions.

Reagents to Be Supplied by User

100% ethanol

Precautions

1. After obtaining the sample, fix the sample in 4%-10% formalin 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 100% ethanol to 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 56°C water bath.

6. Before starting the experiment, preheat the water bath or constant temperature mixer to 56°C, and the centrifuge to 25°C.
7. If downstream experiments need to reduce the low frequency C>T | G>A transition (artificial mutation) to minimize the risk of false positives, 7 μL UNG (1U/uL) (CW0951S) can be added after incubation at 90°C for 1 hour.).

Protocol

1. Sample processing:

   1a. Paraffin-embedded samples: Trim off the excess paraffin in the tissue block with a scalpel to expose the tissue and cut into 5-10 μm thin slices. Take about 1×1 cm^2 slices (about 4-5 slices in total) and put them in a centrifuge tube (self-provided), add 160 μL Buffer DS, vortex for 10 seconds, then add 180 μL Buffer GTL and 20 μL Proteinase K, vortex and shake for 10 seconds. Centrifuge at 12,000 rpm, 25°C for 1 minute.

   Note: 1) If the surface of the sample has been exposed to the air, please discard the 2-3 pieces exposed to the air.

   2) DS will solidify below 18°C, if solidification does not affect the following experiments.

   1b. Samples in formalin fixative: take 20 mg samples, cut them into small pieces, place them in a centrifuge tube, add 500 μL of 10 mM PBS (pH 7.4), vortex and centrifuge at 12,000 rpm for 1 minute. Discard the supernatant and repeat 3 times. Add 180 μL Buffer GTL, 20 μL Proteinase K and vortex to mix.
2. Incubate at 56°C for 1 hour until the sample is completely dissolved. Incubate at 90°C for 1 hour. Centrifuge at 12,000 rpm at 25°C for 1 minute. Use a pipette to carefully pipette the lower aqueous phase (about 180 μL) along the tube wall into a new centrifuge tube, avoiding the suction of the sediment at the bottom of the tube and the upper wax liquid.

   Note:1) The sample incubated at 56°C can be placed at room temperature until the temperature of water bath or dry bath reaches 90°C before incubating at 90°C.

   2) Optional step: Add 7 μL UNG (1U/μL) (Cat. No. : 0951S), 50°C, 5 min, no concussion.
3. 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.
4. Add 20 μL Proteinase K, incubate at 65°C, 450 rpm for 15 min.