FOREGENE
EXPERT IN NUCLEIC ACID EXTRACTION

FFPE DNA Isolation Kit Instruction

Description

This kit is dedicated to extract and purify high-quality genomic DNA from formaldehyde-fixed tissue or paraffin-embedded tissue.

The genomic DNA of the paraffin-embedded sample will be degraded due to the storage time or the environment, making it difficult to extract the genomic DNA. This kit adopts a unique optimized formula, which is suitable for extracting genomic DNA from paraffin-embedded samples with a high extraction success rate. The whole process does not need to use toxic phenol, chloroform or isopropanol precipitation.

This kit uses a spin column that can specifically bind DNA, a brand-new Foregene Protease Plus, and a unique buffer system, which can extract high-quality paraffin-embedded tissue genomic DNA within 2-5 hours.

Features

◆ No RNase contamination: The DNA-Only Column provided by the kit makes it possible to remove RNA from genomic DNA without adding RNase during the experiment, avoiding the laboratory from being contaminated by exogenous RNase.

◆ Fast speed: Foregene Protease Plus has higher activity than similar proteases, and it digests tissue samples faster; the operation is simple, and the genomic DNA extraction operation can be completed within 2-5 hours.

Kit Application

It is suitable for extraction and purification of genomic DNA from formaldehyde-fixed samples and paraffin-embedded tissue samples.

Genomic DNA Application

The genomic DNA purified by the FFPE DNA Isolation Kit has high purity and can be used for routine molecular biology operations, such as: restriction enzyme digestion, PCR, Southern hybridization, library construction and other experiments.

Quality Control

According to FOREGEN's Total Quality Management System , each batch of paraffin-embedded tissue genomic DNA extraction kit is strictly tested for multiple times to ensure the reliability and stability of the quality of each batch of kits..

Kit contents

FFPE DNA Isolation Kit			
Kit contents	DE-05411	DE-05412	DE-05413
	50 T	100 T	250 T
Buffer FL1	20ml	40ml	100ml
Buffer FL2*	20ml	40ml	100ml
Buffer PW*	30ml	60ml	150ml
Buffer WB	25ml	50ml	125ml
Buffer EB	12.5ml	25ml	65ml
Foregene Protease Plus	1.25ml x2	5ml	12.5ml
DNA-Only Column	50 sets	100 sets	250 sets
IFU	1 piece	1 piece	1 piece

*: Buffer FL2 and Buffer PW contain irritating chaotropic salts. Please wear gloves and take relevant protective measures during operation.

Product INFO

Model	Spin-column type	Purification components	Foregene Spin column, reagent
Flux	1-24 samples	Preparation time	2-5h(24 samples)
Centrifuge	Table centrifuge	Tissue hydrolysate separation	centrifugal separation
Liquid volume of spin column	800μl	Purification column DNA load carrying capacity	30μg
Elution volume	50-100μl	Tissue sample processing volume	10-50mg

Storage conditions

◆ This kit can be stored for 12 months under dry conditions at room temperature (15-25°C); if it needs to be stored for a longer period of time, it can be stored at 2-8°C.

Note: If stored at low temperature, the solution is prone to precipitation. Before use, be sure to put the solution in the kit at room temperature for a period of time. If necessary, preheat it in a 37°C water bath for 10 minutes to dissolve the precipitate, and mix it before use.

◆ Foregene Protease Plus solution has a unique formula that is active when stored at room temperature for a long time (3 months); it is more active and stable when stored at 4°C, so it is recommended to store it at 4°C, remember not to keep it at -20°C.

Kit components

◆ Buffer FL1: Provides an environment for enzymatic hydrolysis of tissues.

◆ Foregene Protease Plus: Enzymatically digest tissue samples in the environment of Buffer FL1.

◆ Buffer FL2: Inactivate Foregene Protease Plus and provide a DNA loading environment.

◆ Buffer PW: Remove impurities such as protein and RNA in DNA.

◆ Buffer WB: Remove residual salt ions in DNA.

◆ Buffer EB: elute the DNA on the purification column membrane.

◆ DNA-only Column: specifically adsorb the genomic DNA in the lysate.

DNA-Only Column Characters

Maximum binding capacity	30μg
Maximum loading volume	800μl
Longset DNA fragment	23kb
Minimum elution volume*	50μl
Selection of samples	Paraffin section, formaldehyde-fixed tissue
Maximum amount of starting material*	10-50mg

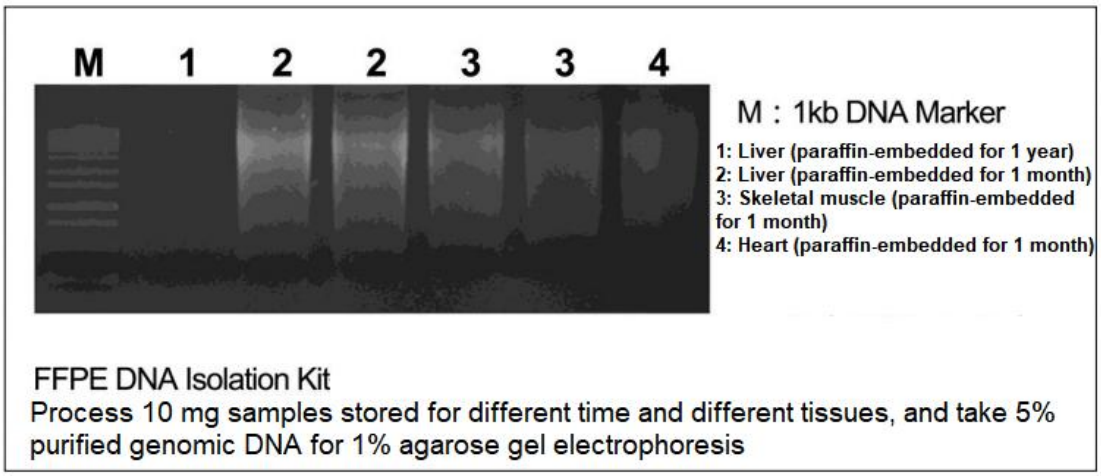
*: The minimum elution system of 50μl is a reasonable recommended volume considering the DNA recovery rate and concentration. If you want to increase the yield of DNA, you can appropriately increase the volume of the eluate; if you want to increase the concentration of purified DNA, while sacrificing part of the DNA yield, reduce the volume of the eluate appropriately, such as using a 30μl elution system. In order to get a higher concentration of DNA.

Genomic DNA extraction yield and purity

The extraction rate of genomic DNA from paraffin-embedded tissue is related to the source of the tissue, storage conditions, storage time, dosage and other factors. The purity of the obtained genomic DNA meets the routine molecular biology experiment operations, and OD260/280 is 1.7-1.9.

Genomic DNA fragment size

The paraffin-embedded tissue genomic DNA extraction kit uses a silica membrane column to separate and purify genomic DNA from a variety of sample sources. The purified genomic DNA fragments will be distributed in a large range according to the storage method and time, and become diffuse bands. See the example below (for reference only).



Notes: (Please read the notes carefully before using the kit)

◆ The sample should avoid repeated freezing and thawing, otherwise the extracted DNA fragments will be smaller and the extraction volume will also decrease.

◆ Before use, carefully check whether there is any precipitation in Buffer FL1, Buffer FL2 and Buffer PW. If there is precipitation, please dissolve it at 37°C and mix it before use.

◆ Before using the kit, be sure to check whether Buffer WB is added with absolute ethanol according to the instructions. Add 60ml of absolute ethanol (DE-05411), 120ml of absolute ethanol (DE-05412), and 300ml of absolute ethanol (DE-05413) to Buffer WB before use.

◆ Elution volume: Buffer EB should not be less than 50 μl, otherwise it will affect the DNA yield.

◆ No need to add RNase to Buffer.

◆ All centrifugation steps are centrifugation at room temperature (15-25℃) in a benchtop centrifuge.

◆ All experimental steps are carried out at room temperature (15-25℃).

Preparation before operation

Please read the instructions carefully before using this kit. The FFPE DNA isolation kit is simple, convenient and fast to operate, and the instructions provide complete information and proper use of the entire kit. Please prepare necessary experimental materials and equipment before use.

Experimental Materials and Equipment

Tissue from multiple sources: paraffin-embedded tissue preserved for many years, formaldehyde-fixed tissue.

Make 1.5ml or 2ml sterile centrifuge tubes.

Make a benchtop centrifuge ($\geqslant$ 13,400 $\times$ g), a 65° C water bath or metal bath, a pipette, a vortexer, etc.

Self-provided reagents

Xylene

Anhydrous ethanol

Safety

This product is only for scientific research use, please do not use it for medicine, clinical medicine, food and cosmetics.

When using chemicals, wear appropriate lab coats, gloves, safety glasses, etc.

Buffer FL2, Buffer PW contain guanidine salts: denaturant, irritant.

BufferWB contain absolute ethanol: Flammable.

Foregene Protease Plus: Sensitizer, Irritant.

Operation guide

The FFPE DNA isolation kit provides a method for rapidly processing formaldehyde-fixed or paraffin-embedded tissue samples and purifying genomic DNA. Please strictly follow the paraffin-embedded tissue genomic DNA extraction procedure for relevant experiments.

Material access instructions

Formaldehyde-fixed or paraffin-embedded tissue samples: 10-50 mg.

Prevent cross-contamination between samples

To prevent cross-contamination between samples during sampling and purification, the following measures can be taken:

During the sampling process, it should be ensured that non-contamination sampling equipment and disposable centrifuge tubes are used for each individual sample.

When aspirating the sample or solution, be careful and gentle to avoid splashing the solution.

After the centrifuge tube is shaken, it should be centrifuged for a short time, and the cap should be opened after the liquid at the mouth of the tube is removed.

Gloves should be worn during the entire operation. If gloves come into contact with samples and their solutions, they should be changed immediately.

Operation steps (Please strictly follow these operation instructions for relevant experimental operations)

Please add anhydrous ethanol to Buffer WB before use. Please refer to the label on the bottle for the added volume.

A. Pretreatment steps of paraffin-embedded tissue samples

1. Use a scalpel to cut off the excess paraffin in the tissue block, take a **10-50mg** paraffin-embedded tissue block sample, cut it into small pieces or 5-10μm sections, and put it into a 1.5ml centrifuge tube.

Note: If part of the tissue in the paraffin block is exposed to air for a long time, 1-2 slices of the surface should be sectioned

2. Add **1.2 ml** of xylene.

a Paraffin section: vortex thoroughly for 10s.

b Paraffin tissue block: fully vortexed and mixed, then placed at 37°C for 30min.

3. Centrifuge at 13,300rpm (~ 17,000×g) for 2 minutes, and remove the supernatant with a pipette. Do not aspirate or shake the pellet.

Note: If paraffin remains on the tube wall after centrifugation, add 1.2ml of xylene again and mix well before centrifugation.

4. Add 1.2ml of anhydrous ethanol and mix well. Centrifuge at 13,300rpm (~17,000×g) for 2 min, and remove the supernatant with a pipette, do not aspirate or shake the pellet.

5. Open the cap of the centrifuge tube and place it in a metal bath at 65°C for 5-10 minutes until the ethanol evaporates completely. Next follow step **B** to perform paraffin tissue genomic DNA extraction.

B. Paraffin tissue genomic DNA extraction steps

1. Add **300 μl** Buffer FL1 and **40 μl** Foregene Protease Plus to the centrifuge tube containing the sample processed in step A and vortex to mix.

2. Place the centrifuge tube in a 65°C metal or water bath and digest the tissue sample as described below.

a Paraffin section: digest for about 1 hr, vortex and mix every 20 min.

b Paraffin blocks: Digest for 2-5hrs, vortexing every 1hr in between.

Note: The vortexing time should not be too long, 2 seconds each time, long-term vigorous vortexing will cause genomic DNA broken into small fragments. If there are still some insoluble solids or impurities after 5hr of enzymatic hydrolysis, proceed directly to the next step.

3. After the enzymatic hydrolysis is completed, add **300 μl** of Buffer FL2, invert and mix well, and place it in a metal bath or water bath at 65° C for 10 minutes.

4. Centrifuge at 12,000 rpm (~13,400×g) for 5 min.

5. Pipette **600 μl** of supernatant into a new 2ml centrifuge tube.

Note: When sucking the supernatant, avoid the sediment from being sucked in. If there are many solid impurities in the sucked supernatant,Step 4 can be repeated once.

6. Add **150 μl** of anhydrous ethanol, shake vigorously until fully mixed, and flocculent precipitation may appear at this time.

7. Put the spin column into the collection tube, transfer the above solution and flocculent precipitate to the spin column (DNA-Only Column), centrifuge at 12,000rpm (~13,400 ×g) for 1min, and discard the waste liquid in the collection tube.

8. Put the spin column back into the collection tube, add **500 μl** Buffer PW to the spin column, centrifuge at 12,000rpm (~ 13,400×g) for 1 min, and discard the waste liquid in the collection tube.

9. Put the spin column back into t`he collection tube, add **700 μl** Buffer WB to the spin column, centrifuge at 12,000rpm (~ 13,400×g) for 1 min, and discard the waste liquid in the collection tube.

10. Repeat step 9 one more time.

11. Put the spin column back into the collection tube and centrifuge the empty tube at 12,000rpm (~ 13,400×g) for 2 minutes.

12. Transfer the spin column to a new 1.5ml centrifuge tube, and drop 50μl of Buffer EB preheated at 65°C into the center of the membrane (do not add the eluent to the press ring, otherwise a large volume will be lost. eluate), placed at room temperature for 5 min, and centrifuged at 12,000 rpm (~13,400 × g) for 1 min. Drop 50μl of pre-warmed Buffer EB into the center of the membrane again, and centrifuge at 12,000rpm (~13,400×g) for 1min. The eluates from the two collections were combined.

Note: If you want to increase the concentration of DNA, you can add the solution from the first centrifugation back to the spin column,Centrifuge at 12,000rpm (~13,400×g) for 1 min.

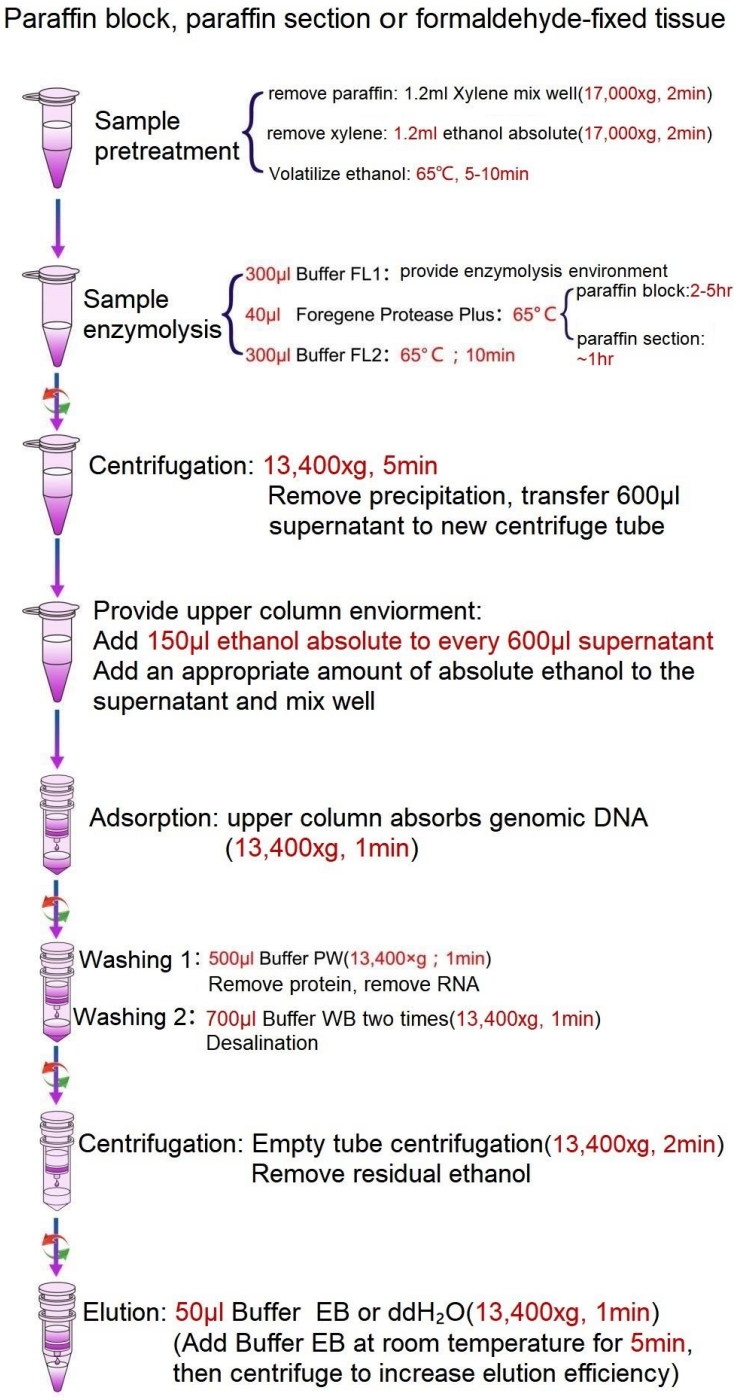
DNA concentration and purity testing

◆The quality of the resulting genomic DNA is related to a number of factors during manipulation. DNA concentration and purity can be checked by agarose gel electrophoresis and UV spectrophotometer.

◆ An OD260 value of 1 for DNA corresponds to approximately 50 μg/ml of double-stranded DNA.

◆The OD260/280 of DNA ≈ 1.7-1.9. If the elution buffer Buffer EB is not used for elution, but deionized water is used, the ratio will be lower, because the pH value and the presence of ions will affect the light absorption value, but it does not indicate low purity.

Work Flow:



Problem Analysis Guide

The following is an analysis of the problems that may be encountered in the extraction of genomic DNA from paraffin-embedded tissues, hoping to help your experiments. In addition, we have dedicated technical support to help you with other experimental or technical problems beyond the operating instructions and problem analysis. If you have any needs, please contact us: 028-83360257 or E-mali: Tech@foregene.com.

Low yield or no DNA

1. The amount of paraffin-embedded tissue samples is too small.

Suggestion: For tissue samples that have been stored for too long or have severe genomic DNA degradation, the amount of tissue samples can be appropriately increased, but it should not exceed 50 mg.

2. The sample was not pre-processed or was not pre-processed thoroughly.

Recommendation: Paraffin-embedded tissue samples should be thoroughly pretreated to prevent residual paraffin or other protease inhibitors from interfering with the enzymatic hydrolysis reaction.

3. The activity of Foregene Protease Plus is reduced or inactivated.

Suggestion: Please confirm the storage conditions of Foregene Protease Plus or replace it with a new Foregene Protease Plus for enzymatic hydrolysis.

4. Buffer WB has no ethanol added.

Recommendation: Please confirm that the correct volume of anhydrous ethanol is added to Buffer WB.

5. The spin column was over-dried before adding the eluent.

Suggestion: The time of opening the cap of the spin column and placing it in the metal bath should not exceed 10 minutes, otherwise it will be difficult to elute the DNA.

6. The eluent was not properly dropped onto the silica mold.

Suggestion: drop the preheated eluent at 65° C into the middle of the silica gel mold, and leave it at room temperature for 5 minutes to increase the elution efficiency.

Extracted genomic DNA with low purity

The low purity of genomic DNA will lead to the failure or unsatisfactory effect of downstream experiments, such as: the enzyme cannot be cut, and the target gene fragment cannot be obtained by PCR.

1. Impurity protein contamination, RNA contamination.

Analysis: The column was not washed with Buffer PW; the column was washed with Buffer PW without the correct centrifugation speed.

Suggestion: Try to ensure that there is no precipitation in the supernatant when the supernatant is passed through the column; be sure to wash the purification column with Buffer PW according to the instructions, and this step cannot be omitted.

2. Impurity ion pollution.

Analysis: The Buffer WB wash column was omitted or only washed once, resulting in residual ionic contamination.

Recommendation: Be sure to wash twice with Buffer WB according to the instructions to try to remove residual ions.

3. RNase contamination.

Analysis: Exogenous RNase was added to the Buffer; incorrect washing operation in Buffer PW resulted in residual RNase and affected downstream RNA experimental operations, such as in vitro transcription, etc.

Suggestion: Foregene nucleic acid extraction kits series can remove RNA without adding RNase, all reagents in FFPE DNA Isolation Kit do not need to add RNase; be sure to wash the purification column with Buffer PW according to the instructions, and this step cannot be omitted.

4. Ethanol residues.

Analysis: After washing the column with Buffer WB, the empty tube centrifugation was not performed.

Recommendation: Follow the instructions for proper empty tube centrifugation.

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