FOREGENE
EXPERT IN NUCLEIC ACID EXTRACTION

Buccal Swab/FTA Card DNA Isolation Kit Instruction

Description

This kit provides an efficient and rapid method to obtain high-concentration genomic DNA from buccal swabs and FTA Card (blood spots). Using our company’s unique DNA-only silica membrane spin column and formula, combined with Foregene Protease, high-concentration, high-quality genomic DNA can be extracted in 80 minutes. The specially designed small purification column binds genomic DNA, and the DNA can be eluted with a small amount (15μl) elution system to increase the concentration of the obtained genomic DNA, which is convenient for downstream detection or experiment. The kit can process one or more samples at a time, and the purification process does not require extraction of organic substances such as phenol, chloroform, and time-consuming isopropanol or ethanol precipitation, and the operation is simple and time-saving.

The DNA-only silica gel membrane used in the spin column of this kit is a unique new material of the company, which efficiently and specifically adsorbs DNA, and can remove RNA, impurity proteins, ions and other organic compounds in cells to the greatest extent. The obtained DNA fragments are large, with high purity, stable and reliable quality, and the maximum carrying capacity of a spin column is 80 μg DNA. The obtained DNA can be used for molecular biology experiments such as restriction digestion, PCR, Southern hybridization, library construction and so on.

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 has higher activity than similar proteases, digests samples quickly; simple operation.

◆ Convenient: The centrifugation is performed at room temperature, and there is no need for 4°C low-temperature centrifugation or ethanol precipitation of DNA.

◆ Safety: No organic reagent extraction is required.

◆ High quality: The extracted genomic DNA has large fragments, no RNA, no RNase, and extremely low ion content, which can meet the requirements of various experiments.

◆ Micro-elution system: It can increase the concentration of genomic DNA, which is convenient for downstream detection or experiment.

Kit Application

It is suitable for purification of genomic DNA from the following samples: buccal swabs, FTA Card (blood stains).

Genomic DNA Application

The genomic DNA purified by the Buccal Swab/FTA Card 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 FOREGENE's Total Quality Management System , each batch of Buccal Swab/FTA card DNA isolation kit is strictly tested for multiple times to ensure the reliability and stability of the quality of each batch of kits.

Kit contents

Buccal Swab/FTA Card DNA Isolation Kit			
Kit contents	DE-05811	DE-05812	DE-05813
	50 T	100 T	250 T
Buffer ST1	18ml	36ml	90ml
Buffer ST2	18ml	36ml	90ml
Linear Acrylamide	250μl	500μl	1.25ml
Buffer PW	25ml	50ml	125ml
Buffer WB	25ml	50ml	125ml
Buffer EB	10ml	20ml	50ml
Foregene Protease	1.25ml	2.5ml	5ml
DNA-Only Column	50 sets	100 sets	250 sets
IFU	1 piece	1 piece	1 piece

Product INFO

Model	Spin-column type	Purification components	Foregene Spin column, reagent
Flux	1-24 samples	Preparation time	80min (24 samples)
Centrifuge	Table centrifuge	Tissue hydrolysate separation	centrifugal separation
Purification column DNA load carrying capacity	30μg	Liquid volume of spin column	800μl
Elution volume	15-30μl	Sample processing volume volume	1 buccal swab 1-3 slices of blood with a diameter of 3mm

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 place 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 solution has a unique formula, which is active when stored at room temperature for a long time (3 months); its activity and stability will be better 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 ST1: Provides an environment for enzymatic digestion of tissue samples.

◆ Foregene Protease: Enzymatically digest tissue samples in the environment of Buffer ST1.

◆ Buffer ST2: Inactivate Foregene Protease and provide a DNA loading environment.

◆ Linear Acrylamide: Reduce nucleic acid background adsorption and improve DNA elution efficiency.

◆ 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: Specific adsorption of genomic DNA in the lysate.

DNA-Only Column Characters

Maximum binding capacity	30μg
Maximum loading volume	800μl
Longset DNA fragment	23kb
Minimum elution volume*	15μl
Selection of samples	Buccal Swab，blood stains
Maximum amount of starting material*	1 buccal swab, 1-3 blood stains

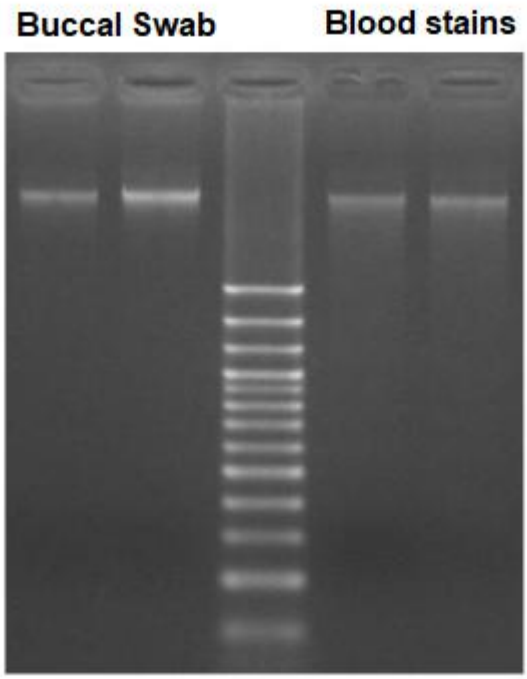
*: The minimum elution system of 15μl is a reasonable recommended volume considering DNA recovery and concentration. If you want to increase the yield of DNA, you can appropriately increase the volume of the eluate; if you want to increase the concentration of purified DNA, at the expense of part of the DNA yield, appropriately reduce the volume of the eluate, in order to obtain a higher concentration of DNA.

Genomic DNA extraction yield and purity

The yield of genomic DNA in a small sample is related to the source of the sample, storage conditions, degree of enzymatic hydrolysis, and operation. The Buccal Swab/FTA Card DNA isolation Kit provides an effective method to obtain genomic DNA from a small sample, generally a buccal swab 0.5-3μg of genomic DNA can be obtained (related to the number of cells contained in the buccal swab); a blood spot with a diameter of 3mm can obtain 0.2-0.5μg of genomic DNA. The obtained genomic DNA has high purity, and OD260/280≈1.7-1.9.

Genomic DNA fragment size

The Buccal Swab/FTA Card DNA isolation Kit uses a silica membrane column to isolate and purify genomic DNA from a variety of sample sources. The purified genomic DNA fragments size are all around 23 kb. See below:



Extract 1 buccal swab and 3 genomic DNA with a diameter of 3mm blood stains. Two replicates of each sample are eluted according to 30μl Buffer EB, 5μl of gene DNA and 100bp DNA Ladder are coagulated in 1% agarose Gel electrophoresis.

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

◆ One buccal swab is sufficient for each purification system. For multiple buccal swabs, please use multiple purification columns for purification.

◆ For single purification, it is recommended to use 1-3 FTA Cards with a diameter of about 3mm for experiments. If the blood card storage quality is not good, increase the usage amount.

◆ Before use, carefully check whether there is any precipitation in Buffer ST1, Buffer ST2 and Buffer PW. If there is precipitation, please dissolve it at 37° C and mix well before use.

◆ Before using the kit, be sure to check whether Buffer WB has been added with absolute ethanol according to the instructions. Add 60ml of absolute ethanol (DE-05811), 120ml of absolute ethanol (DE-05812), and 300ml of absolute ethanol (DE-05813) to Buffer WB before use.

◆ During the lysis of the sample, the sample should always be immersed in the lysis buffer. If the sample adheres to the cap and inner wall of the tube, it can be processed by short centrifugation.

◆ Elution volume: The use of a micro-elution system will increase the DNA concentration, but the Buffer EB should not be less than 15 μl, otherwise it will affect the DNA yield.

◆ Remember not to add RNase to any buffer.

◆ All centrifugation steps are centrifugation at room temperature (15-25°C) using 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 oral swab/blood card genomic DNA extraction kit is simple, convenient and fast to operate, and the instructions provide complete information and correct usage of the entire kit. Please prepare necessary experimental materials and equipment before use.

Experimental Materials and Equipment

◆ Single purification system: a buccal swab or 1-3 blood spots with a diameter of about 3mm.

◆ 1.5ml or 2ml sterile centrifuge tube.

◆ Desktop centrifuge (≥13,400×g), 65℃ water bath or metal bath, pipette, vortexer, etc.

Self-provided reagents

◆ Anhydrous ethanol

Safety

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

◆ Wear suitable lab coats, gloves, safety glasses,etc. when working with chemicals.

◆ Buffer ST2 contains guanidine salts: denaturant, irritant.

- Buffer PW contains guanidine salts: denaturant, irritant.
- Buffer WB contains absolute ethanol: Flammable.
- Foregene Protease: sensitizer, irritant.

Operation guide

Oral swab/blood card genomic DNA extraction kit provides a method to quickly process oral swabs, blood cards or blood spots and purify genomic DNA. Please strictly follow the instructions for genomic DNA extraction.

Material access instructions

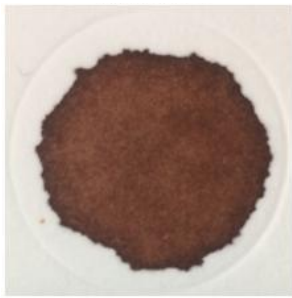
Oral swab:

- Oral swab sampling method: Use sterile oral swabs to wipe the cheeks repeatedly 10-15 times.
  Note: To ensure that the sample is not contaminated by food or drink, do not eat or drink within 30 minutes before sampling.
- Post-processing of oral swab sampling: Use scissors to cut off the cotton swab on the oral swab from the rod and place it in a 2ml centrifuge tube for later use.

FTA Card(blood spots):

- Preparation of blood spot samples: Gently mix the anticoagulant tube containing blood; take 70-80μl of anti-coagulated whole blood with a pipette, and slowly drop the blood on the center of the sampling circle on the FTA Card (d=2.5cm). Add to the circle on the blood card, taking care to avoid air bubbles. Air dry the FTA Card on a dry, clean, flat, non-absorbent table or professional paper drying rack.
  Note: Do not place in the sun to dry or artificially accelerate drying.
- Quality control and validity judgment of blood spot samples: Qualified blood spots should meet the requirements of 70-80μl blood samples to fill the sample collection circle on the FTA Card as much as possible, the blood samples to fully soak the blood card, the blood spots without fading and pollution, no serum rings, blood spots not layered. Qualified blood spots are shown in the following figure:

Qualified blood spots



- Processing after collection of blood spot samples: Cut blood spots and place them in a clean 2ml centrifuge tube for later use.

Operation steps (please carry out relevant experimental operations in strict accordance with this operation instruction)

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

1. Cut off a cotton swab from a sample, and place it in a clean 2ml centrifuge tube or place 1-3 pieces of blood spots with a diameter of about 3mm (see Page 10 for the preparation method) in a clean 2ml centrifuge tube.

2. Add **300μl** Buffer ST1 to the centrifuge tube and mix by vortexing.

3. Add **20μl** Foregene Protease to the above system, and mix by vortexing for 5 sec. Briefly centrifuge to collect droplets adhering to the cap and inner wall of the centrifuge tube.

4. Place the centrifuge tube in a metal bath or water bath at 65℃ for 1 hour, and vortex and mix for 5 seconds at 15 minute intervals.

5. Add **310μl** Buffer ST2, and after mixing, add **4μl** Linear Acrylamide, vortex for 5 sec, and centrifuge briefly to collect the droplets attached to the cap and inner wall of the centrifuge tube.

6. Place the centrifuge tube in a metal bath or a water bath at 65℃ for 10 minutes, shake and mix once every 3 minutes, and centrifuge briefly to collect the droplets attached to the cap and inner wall of the centrifuge tube.

7. Discard the oral swab in the solution, add **200μl** of absolute ethanol, vortex and mix for 5 sec, and flocculent precipitation may appear at this time.

8. Transfer the solution and flocculent precipitate obtained in the previous step to a DNA-Only Column, centrifuge at 12,000 rpm (~13,400 ×g) for 1 min, and discard the waste liquid in the collection tube.

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

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

11. Repeat step 10 once.

12. Put the spin column back into the collection tube and centrifuge the empty tube at 12,000 rpm (~13,400 ×g) for 1 min.

13. Transfer the spin column to a new 1.5ml centrifuge tube, and drop **15-100μl** of Buffer EB preheated at 65℃ into the center of the membrane (do not add the eluent to the press ring, otherwise a large volume of eluate will be lost), placed at room temperature for 5 min, and centrifuged at 12,000 rpm (~13,400 ×g) for 1 min.

Note: The volume of Buffer EB should not be less than 15μl. Within the recommended elution volume range, increasing the volume of Buffer EB appropriately can improve the DNA yield. If you want to increase the DNA concentration, add the solution from the first centrifugation back to the spin column and centrifuge at 12,000 rpm (~13,400 ×g) for 1 min.

DNA concentration and purity detection

- The quality of the obtained genomic DNA is related to a variety of factors during the 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.
- 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 mean that the purity is low.

Problem Analysis Guide

The following analysis of possible problems in Buccal swab/FTA card DNA extraction is helpful to your experiment. In addition, for other experimental or technical problems in addition to operating instructions and problem analysis, we have dedicated technical support to help you. If you have any needs, please contact us at: 028-83360257 or E-mali:Tech@foregene.com.

The purification column is clogged

In this kit, in the genomic DNA extraction operation, the purification column is directly adsorbed on the sample enzymatic lysis mixture without centrifugation step, and the purification column may be blocked due to incomplete enzymization and high viscosity of the sample.

The following possible causes are as follows:

1. Incomplete enzymatic digestion of tissue samples.

Recommendation: The sample processing time of Foregene Protease can be appropriately extended or the supernatant can be taken after centrifugation at 12,000 rpm (~13,400 × g) for 5 min.

2. Excessive use of tissue samples or large tissues.

Recommendation: It is best not to exceed 1 Buccal swab in the sample; if the sample is too large, increase the dosage of Buffer ST1, Foregene Protease, buffer ST2 accordingly.

3. The sample viscosity is too high.

Recommendation: Samples can be appropriately diluted with 10 mM of Tris-HCl before genomic DNA extraction.

4. Fragments of the blood card have been sucked.

Recommendation: The transient centrifugation time of step 6 of blood spot (FTA Card) genomic extraction can be appropriately extended.

Low yield or no DNA

There are often a variety of factors that affect genomic DNA yield, including sample origin, sample storage conditions, sample preparation, manipulation, etc.

Genomic DNA cannot be obtained during extraction

The possible causes are as follows:

1. Improper preservation of samples or storage for too long leads to genomic DNA degradation.

Recommendation: Oral swabs should preferably be freshly sampled, and it is not

advisable to use preserved swabs for genomic DNA extraction operations; blood spot samples should ensure that the quality is qualified and the storage time should not be too long.

2. Too little tissue usage may result in no extraction of the corresponding genomic DNA.

Recommendation: Follow the buccal swab sampling instructions in the operation guide, and wipe as many times as possible so that enough cells can be attached to the oral swab for genomic DNA extraction; for blood spot sample extraction, the blood spot cutting area can be appropriately increased.

3. Foregene Protease is improperly preserved, resulting in a decrease in its activity or inactivation.

Recommendation: Confirm the storage conditions of the Foregene Protease or replace it with a new Foregene Protease for enzymatic reaction.

4. Improper preservation of the kit or storage time is too long, resulting in the failure of some components in the kit.

Recommendation: Purchase a new Buccal swab DNA isolation kit for related procedures.

5. Buffer WB does not add absolute ethanol.

Recommendation: Confirm that buffer WB adds the correct volume of absolute ethanol.

6. The eluent is not correctly added to the silicone film.

Recommendation: Add 65 °C pre-warmed eluent drops to the middle of the silicone membrane and leave at room temperature for 5 min to increase the elution efficiency.

Low-yield genomic DNA isolated

The following possible causes are as follows:

1. Improper preservation of samples or storage for too long leads to genomic DNA degradation.

Recommendation: Oral swabs are preferably freshly sampled, and preserved swabs should not be used for genomic DNA extraction.

2. If the amount of tissue sample is too small, the extracted genomic DNA content will be less.

Recommendation: Follow the oral swab sampling instructions in the operating guide, wiping as many times as possible so that enough cells can be attached to the oral

swab for genomic DNA extraction.

3. Foregene Protease is improperly preserved, resulting in a decrease in its activity or inactivation.

Recommendation: Confirm the storage conditions of the Foregene Protease or replace it with a new Foregene Protease for enzymatic reaction.

4. Eluent problems.

Recommendation: Use Buffer EB for elution; if using ddH_2O or other eluents, confirm that the pH of the eluate is between 7.0-8.5.

5. The eluate is not correctly added dropwise.

Recommendation: Add eluent drops to the middle of the silicone membrane and leave at room temperature for 5 min to increase elution efficiency.

6. The elution liquid accumulates too little.

Recommendation: Use eluent for genomic DNA elution as required in the instructions, at least no less than 15 μl.

Low purity of genomic DNA isolated

Low genomic DNA purity can lead to failure or unsatisfactory results of downstream experiments, such as: enzymes cannot be cut open, PCR can not get the gene fragment of interest, etc.

The possible causes are as follows:

1. Heteroprotein pollution, RNA pollution.

Analysis: The purification column was not washed using Buffer PW; the Buffer PW wash purification column was not washed using the correct centrifugal speed.

Recommendation: Ensure that there is no precipitation in the supernatant before adding ethanol; be sure to wash the purification column according to the instructions, and this step cannot be omitted.

2. Impurity ion pollution.

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

Recommendation: Be sure to wash Buffer WB 2 times as directed to remove residual ions as much as possible.

3. RNA enzyme contamination.

Analysis: Foreign RNases were added to buffer; Buffer PW wash operation was

incorrect, resulting in RNase residues, affecting downstream RNA experimental operations, such as in vitro transcription.

Recommendation: Foregene series nucleic acid isolation kits can remove RNA without additional addition of RNase,thus buccal Swab/FTA Card DNA Isolation Kit needn't add RNase; be sure to follow the instructions for Buffer PW washing purification column, and this step cannot be omitted.

4. Ethanol residue.

Analysis: Buffer WB did not perform empty tube centrifugation after washing the purification column.

Recommendation: Perform the correct empty tube centrifugation operation according to the instructions.

5. Other impurity pollution.

Analysis: Saved samples or special samples are not pretreated.

Recommendation: Thoroughly pretreat the sample as instructed.

中国●福际 World's Foregene

Foregene Co., Ltd
Tel: 86-28-83360257，86-28-83361257
E-mail：info@foregene.com
Http://www.foregene.com