Extiquick™ FFPE DNA Kit

Operating instruction

(Cat#EX003,Version1.3)

欣基（杭州）生物科技有限公司
WisGen BioSciences Co., Ltd

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Overview

Formalin-fixed and paraffin-embedded (FFPE) tissue samples are routinely prepared from human surgical tissue samples allowing to store them in archives for years. The Extiquick™ FFPE DNA Kit is designed for fast and convenient purification of DNA from various amounts of archived FFPE tissue. Up to 8 sections (10 µm thickness) of FFPE sample from various tissues can be used for genomic DNA extraction without overnight incubation. Elimination of toxic reagents commonly used for deparaffinization (i.e., xylene) allows a safe DNA purification procedure with high DNA yields and excellent quality. Each preparation recovers up to 8 µg of genomic DNA that can be eluted in volumes from 20 µL allowing for concentrated samples. Due to high decrosslinking efficiency high quality purified DNA shows reliable performance in sensitive downstream applications such as qPCR, PCR (amplicons up to 1 kb) or NGS library preparation.

Component

Product	Component	100 preps (EX003-100)	250 preps (EX003-250)	Storage	Expiry date
Extiquick™ FFPE DNA Kit	DPS Buffer	60 mL	150 mL	Room temp	24 months
	Proteinase K	50 mg	120 mg	-30 ℃~-15 ℃	
	Protease Dissolve Buffer	2.5 mL	6 mL	2~8 ℃	
	FFPE Lysis Buffer	40 mL	100 mL	Room temp	
	FFPE Wash Buffer Ⅰ	60 mL	150 mL	Room temp	
	FFPE Wash Buffer Ⅱ	30 mL	75 mL	Room temp	
	Elution Buffer	5 mL	12.5 mL	Room temp	
	Silic Beads B	2 mL	5 mL	2~8 ℃	

Storage conditions

Store at 2 ~ 8 ℃, Adjust the shipping method according to the destination.

Application scope

This product is suitable for FFPE DNA extraction

Self-prepared Material

Magnetic rack (WisGen Bio xMag™ Magnet or other supplier)

Nuclease-free ddH_20 and tube

Ethanol(100%)

Note

For research use only . Not for use in diagnostic procedures .

1. Mix blood samples thoroughly before extraction.
2. Before the first use, add the correct volumes of absolute ethanol to Wash Buffer G I and Wash Buffer G II as indicated on the label of the reagent bottle, and mix them well.
3. Check if there is any precipitation in GD Lysis Buffer and Wash Buffer G I before use. If precipitates have formed, they can be re-dissolved in a 37 ℃ water bath and mixed well before use.
4. Perform all steps at room temperature (15 ~ 25 ℃).
5. Sample input volume: 200 μL for whole blood samples and 100 μL for bone marrow samples.
6. Before using Silic Beads B, please equilibrate at room temperature by removing it from 4 ℃ for 30 minutes in advance, and use it by shaking and mixing.
7. Do not over-dry the beads as this may cause a decrease in DNA elution efficiency.

Experiment Process

Before Usage

Dissolve Proteinase K: Please add Proteinase Dissolve Buffer to dissolve Proteinase K according to the amount indicated on the reagent label, the final concentration is 20 mg/mL, gently invert to let Proteinase K fully dissolve for 10 min. Proteinase K dry powder can be stored at 2 ~ 8 ℃ for one year, while Proteinase K solution should be stored at -30 ~ -15 ℃ to avoid repeated freezing and thawing, which may affect the activity.

Manual operation

Step 1: Sample Lysis

1.1 Blood sample

1.1.1 Gently mix the blood collection tube upside down ten times, and take 200 μL of blood sample into a 1.5 mL centrifuge tube.

1.1.2 Add 2.5 times the volume of blood sample to FLA Buffer, mix upside down for 10 sec, centrifuge at 10,000 rpm for 1 min, remove supernatant and retain leukocyte precipitate.

Note: If the red blood cell cleavage is incomplete, repeat step 1.1.2 once.

1.1.3 Perform the experimental procedure to step 2.

1.2 Cell sample

1.2.1 Transfer the cultured cells to a 1.5 mL centrifuge tube, centrifuge at 1000 rpm for 3 min, remove the medium and retain the cell precipitate.

1.2.2 Add 500 μL of 1×PBS buffer and shake gently to disperse the cell mass.

1.2.3 Centrifuge at 1000 rpm for 3 min, aspirate and discard the supernatant, retain the cell precipitate.

1.2.4 Perform the experimental procedure to step 2.

1.3 Tissue sample

1.3.1 Take <25 mg of fresh or frozen tissue for thorough homogenization, liquid nitrogen grinding, or shredding.

Note: Tissues with high cell content such as liver and spleen can be taken <10 mg.

1.3.2 Transfer the processed tissue to a 1.5 mL centrifuge tube and set aside.

1.3.3 Perform the experimental procedure to step 2.

Step 2: Nucleic acid extraction

2.1 Add 450 μL GD Lysis Buffer, 20 μL Proteinase K (20 mg/mL) to the sample, vortex and mix well.

Note: GD Lysis Buffer should be used after shaking well. If there is precipitate, it can be heated in a water bath at 37 ℃, dissolved completely and mixed well before use.

2.2 Place the sample in a 58 °C shaking metal bath for 30 min ~ 3 h. Mix upside down 3 times during the digestion.

Note:

1) **Lysis time depends on the sample type and tissue fragmentation effect, the recommended lysis time is 30 min for leukocyte precipitation , cultured cells and 1~3 h for tissue samples.**

2) **After sample lysis is complete, if there are obvious undigested impurities in the digestion solution, centrifuge at 10,000 rpm for 3 min to remove the impurities and transfer the supernatant solution to a new 1.5 mL centrifuge tube;**

2.3 After 5 min at room temperature, add 200 μL of isopropanol and shake and mix for 10 sec.

Note: If flocs or jelly-like colloids appear after adding isopropanol, be sure to remove them with a pipette tip before adding the beads to avoid adhesion to the beads and reduce the DNA yield.

2.4 Add 20 μL of Silic Beads B, shake and mix for 30 sec, place at room temperature for 10 min, shake and mix every 3 min.

Note: Silic Beads B should be left at room temperature for 30 min and vortexed and mixed before use.

2.5 After transient centrifugation, place on a magnetic rack for 2 min. After the solution is completely clarified, carefully remove the supernatant.

2.6 After Remove the centrifuge tube from the magnetic rack, add 800 μL Wash Buffer G I, and shake and mix for 10 sec.

2.7 After transient centrifugation , place on the magnetic rack for 2 min, allow the solution to clarify completely and carefully remove the supernatant.

2.8 After Remove the centrifuge tube from the magnetic rack, add 800 μL Wash Buffer G II, and shake and mix for 10 sec.

2.9 After transient centrifugation, place on the magnetic rack for 2 min, let the solution clarify completely, aspirate all the solution, open the cap and air dry for 1~2 min.

Note: Ethanol residue will affect the subsequent PCR reaction, so make sure the ethanol evaporates completely when drying, and also need to avoid over-drying the magnetic beads, resulting in lower DNA elution efficiency.

2.10 Add 50 μL of Elution Buffer, vortex and mix well, and incubate for 3 min at room temperature.

2.11 Place the centrifuge tube on a magnetic rack for 2 min and transfer the supernatant to a new tube.

2.12 The eluted DNA can be used directly for subsequent experiments or stored at -20 ℃.

Automatic Machine operation

Step 1: Sample Preparation

1.1 Blood sample

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Note: If the red blood cell cleavage is incomplete, repeat step 1.1.2 once.

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1.1.4 Perform the experimental procedure to step 2.

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1.2.2 Add 500 μL of 1×PBS buffer and shake gently to disperse the cell mass.

1.2.3 Centrifuge at 1000 rpm for 3 min, aspirate and discard the supernatant, retain the cell precipitate.

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1.2.5 Perform the experimental procedure to step 2.

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1.3.1 Take <25 mg of fresh or frozen tissue for thorough homogenization, liquid nitrogen grinding, or shredding.

Note: Tissues with high cell content such as liver and spleen can be taken <10 mg.

1.3.2 Transfer the processed tissue to a 1.5 mL centrifuge tube and set aside.

1.3.3 Add 450 μL GD Lysis Buffer, 20 μL Proteinase K (20 mg/mL) to the sample, vortex and mix well.

1.3.4 Perform the experimental procedure to step 2.

Step 2: Sample Lysis

2.1 Referring to the table below, add the reagent to each well of the 96-well plate.

Plate Location	1/7	2/8	3/9	4/10	5/11	6/12
Reagent	Step 1 Product	Wash Buffer G I 800 μL	Wash Buffer G II 800 μL	/	Silic Beads B 20 μL	Elution Buffer 80 μL

Note:

1）**Please try to shake evenly before each aspiration of magnetic beads, in order to avoid too few beads sinking to the bottom and cannot be adsorbed;**

2）**In order to improve the efficiency, it is recommended to use Multi-channel pipette;**

3）**Please use the reagents as soon as possible after the addition of the plate, in order to prevent the alcohol from volatilising and leading to fluctuations in the results.**

2.2 Place the sample-loaded 96-well plate in Auto-Pure 32A Automatic Nucleic Acid Extractor, insert the magnetic sleeve rod, open the instrument operation software and run the "Lysis" program :

Step	Well Location	Name	Mix Time (min)	Magnetisation time (sec)	Waiting Time (min)	Volume (μL)	Mix Speed (1-10)	Temp (℃)
1	1	Lysis	10.0	0	0.0	800	5	95

Step 3: Nucleic acid extraction

1. After lysis, open the door of Auto-Pure 32A and add 200 μL of isopropanol into the 1st/7th column of the 96-well plate.
2. After the deep-well plate is returned to its original position, run the "Whole Blood/Tissue DNA Extraction" program with the following parameters. If the original program is lost, you can set it by yourself.

Step	Well Location	Name	Mix Time (min)	Magnetisation time(sec)	Waiting Time (min)	Volume (μL)	Mix Speed (1-10)	Temp (℃)
1	5	Beads Transform	1.0	60	0.0	100	5	OFF
2	1	DNA Binding	10.0	60	0.0	1000	5	OFF
3	2	Wash Buffer G I	2.0	60	0.0	900	5	OFF
4	3	Wash Buffer G II	2.0	60	0.0	900	5	OFF
5	6	Elution	7.0	60	2.0	80	5	65
6	3	Absorb Beads	1.0	0	0.0	500	5	OFF

Step 4: Nucleic acid transform

After the program is finish, the 6/12 Elution buffer is transferred to a clean EP tube, the deep-well plate and magnetic sleeve are discarded and the extraction process is complete

FAQ & Troubleshooting

Question	Reasons	Solutions
Low DNA Yield	1. Improper storage, repeated freezing and thawing, or prolonged storage of blood samples.	Use fresh blood samples for genomic DNA extraction.
	2. Too little/Too much blood sample used.	Use the sample volume recommended in the Instructions for Use.
	3. Partial or complete activity loss of Proteinase K due to improper storage.	Use new Proteinase K for digestion.
	4. Incomplete sample Lysis.	Ensure that the sample is thoroughly mixed with Proteinase K and Buffer DCL. Extend the lysis time in the 65°C water bath or mix more times by inversion as appropriate.
	5. Over-drying of beads.	Inspect the beads during air-drying to avoid over-drying.
	6. Elution buffer issues.	Please elute with Elution Buffer. If ddH2O or another elution buffer is used, make sure that its pH is between 7.5 - 8.5.
	7. Incomplete elution.	The beads are not thoroughly mixed after the addition of Elution Buffer. Extend the shaking time as appropriate until the beads are thoroughly mixed.
	8. Buffer WA or Buffer WB not supplemented with the correct volume of ethanol absolute.	Add appropriate volumes of absolute ethanol as indicated on the label of the reagent bottle.
Low DNA Purity	1. Too little blood sample used .	See above.
	2. Protein contamination.	Buffer WA wash has been omitted, or only one wash has been performed. Wash twice with Buffer WA in accordance with the Instructions for Use.
	3. Salt ion contamination.	Buffer WB wash has been omitted, or Buffer WB has not been supplemented with the correct volume of absolute ethanol. Add the correct volume of absolute ethanol as indicated on the label of the reagent bottle, and perform the wash step.
	4. Residual ethanol.	The beads have not been properly dried. Extend the air-drying time as appropriate.
Special Samples	1. Cell-rich samples, such as the blood of leukemia patients or cord blood.	Limit the input to 50 - 200 µl and extend lysis time (≥15 min) to ensure complete lysis.
	2. Cell samples.	The input of cells should be less than 1×10^7.

欣基（杭州）生物科技有限公司
WisGen BioSciences Co.,Ltd

Add ： 浙江省杭州市钱塘区福城路 400 号 6 幢 8 层
Service ： order@wisgen.cn Web ： www.wisgen.cn