Extiquick™ cfDNA DNA Kit

(200～600μl plasma/serum)

Operating instruction

(Cat#EX006,Version1.4)

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Not For Use in Diagnostic Procedures.

仅供科研使用

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Overview

Extiquick™ cfDNA DNA Kit consist of highly efficient superparamagnetic hydroxyl-modified particle-size homogenised magnetic beads and a special buffer system, which enables the rapid and efficient extraction of genome-dna free cfDNA from 200 ~ 600μL of plasma, serum, CSF and urine sample. The kit is easy to operate, short process, high yield of free nucleic acid DNA, high purity, stable quality, safe and reliable, especially suitable for the use of automated extraction workstation. It can be used in qPCR, NGS and other applications.

Component

Component	48preps (EX006-048)	96preps (EX006-096)	Storage	Expiry date
Proteinase K	24mg	45 mg	2 ~ 8°C	18 months
Protease Dissolve Buffer	1.2 mL	2.25 mL	2 ~ 8°C	
cfDNA Binding Buffer	19.2mL	38.4 mL	Room Temp	
cfDNA Lysis Buffer	9.6 mL	19.2 mL	Room Temp	
cfDNA Wash Buffer Ⅰ	38.4 mL	76.8 mL	Room Temp	
cfDNA Wash Buffer Ⅱ	8 mL	16 mL	Room Temp	
Elution Buffer	1.44 mL	2.88 mL	Room Temp	
Silic Beads A	960 μL	1.92 mL	2 ~ 8°C	

Storage conditions

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

Application scope

This product is suitable for plasma, serum, CSF and urine cfDNA extraction.

Self-prepared Material

Magnetic rack (WisGen Bio xMag™ Magnet or other supplier, specifically designed for 1.5 mL tubes) Nuclease-free ddH_20 and tube Ethanol (100%) 1X PBS Buffer Non-stick, low-binding, DNase/RNase-free tubes (1.5 mL) Heater (for sample lysis)

Note

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1. Mix blood samples thoroughly before extraction.
2. Before the first use, add the correct volumes of absolute ethanol to cfDNA Wash Buffer Ⅱ as indicated on the label of the reagent bottle, and mix them well.
3. Check if there is any precipitation in cfDNA Lysis Buffer and cfDNA Binding Buffer 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. Before using Silic Beads A, please equilibrate at room temperature by removing it from 4 ℃ for 30 minutes in advance, and use it by shaking and mixing.
6. Do not over-dry the beads as this may cause a decrease in DNA elution efficiency.
7. During the steps of blood sample collection, it is strongly recommended to use cfDNA special blood collection tubes to achieve the best experimental results, and strictly follow the instructions of blood collection tubes for preservation and transport; if conventional blood collection tubes are used, it is recommended to separate serum or plasma samples at the first time and preserve them at -80 ℃, transport them on dry ice, and avoid repeated freezing and thawing.
8. Blood samples collected using blood collection tubes containing heparin anticoagulant should not be used for PCR and second generation sequencing related experiments.

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 pre-processing

1.1. Gently mix the whole blood upside down centrifuge at 1,800 rpm (300× g) for 20 min at room temperature.

1.2. Separate the supernatant 200 μL to 600 μL plasma into a 1.5 mL centrifuge tube.

Note: When taking the upper plasma layer, please be careful not to aspirate the white liquid in the middle layer, and it is recommended to discard the last 500μL~1mL of plasma, so as not to bring in leukocytes and cause contamination; if accidentally aspirated, please re-centrifuge and re-dispense.

1.3. Centrifuge again at 7,900 rpm (6,000×g) for 5 min at room temperature to completely remove residual blood and cells debris. Transform all the supernatant to a 1.5 mL centrifuge tube.

Note: In general, 200 ~ 600 μL of plasma/serum can meet the DNA input requirements for qPCR and NGS library construction, and the plasma/serum input is within the range of 100 ~ 600 μL without changing the amount of other reagents used.

Table 1: Plasma status and solution

Plasma Color	Status	Solution
Light yellow, translucent yellow	Normal	Normal sample ,go to the next step
Light red	Slight haemolysis	Repeat step 1.1 once again，then go to the next step
Milky white	Excessive lipid	Repeat step 1.1 once again，then go to the next step
Red	Severe haemolysis	Repeat step 1.1 once again，If the supernatant color is reddish, go to step 1.2; if the color is still reddish, re-collect sample

Step 2: Nucleic acid extraction

2.1 Add 200 μL cfDNA Lysis Buffer, 20 μL Proteinase K (20 mg/mL) to the sample, vortex and mix well.

Note: cfDNA 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 ℃ shaking metal bath for 25 min. Mix upside down 3 times during the digestion. After digestion ,place the tube at 4℃ for 5 min.

2.3 Add 400 μL cfDNA Binding buffer and 20 μL of Silic Beads A, shake and mix for 30 sec, place at room temperature for 10 min, shake and mix for 10 sec every 3 min.

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

2.4 After transient centrifugation, place on a 1.5 mL magnetic rack for 5 min. After the solution is completely clarified, carefully remove the supernatant.

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

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

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

2.8 After transient centrifugation, place on the magnetic rack for 2 min, let the solution clarify completely, aspirate all the solution, add 200 μL anhydrous ethanol 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 of the tube for 1~2min.

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 32 μL of Elution Buffer, vortex and mix well, and incubate for 5 min at room temperature.

2.11 Place the centrifuge tube on a magnetic rack for 2 min and transfer the 30 μL 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. Gently mix the whole blood upside down centrifuge at 1,800 rpm (300× g) for 20 min at room temperature.

1.2. Separate the supernatant 200 μL to 600 μL plasma into a 1.5 mL centrifuge tube.

Note: When taking the upper plasma layer, please be careful not to aspirate the white liquid in the middle layer, and it is recommended to discard the last 500 μL ~ 1 mL of plasma, so as not to bring in leukocytes and cause contamination; if accidentally aspirated, please re-centrifuge and re-dispense.

1.3. Centrifuge again at 7,900 rpm (6,000× g) for 5 min at room temperature to completely remove residual blood and cells debris. Transform all the supernatant to a 1.5 mL centrifuge tube.

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Step 2: Sample Lysis

2.1 Referring to the table below, add the reagent to each well of the special reagent strip.

Plate Location	1/7	2/8	3/9	4/10	5/11	6/12
Reagent	cfDNA Lysis Buffer + Proteinase K+cfDNA Binding Buffer	/	cfDNA Wash Buffer Ⅰ	cfDNA Wash Buffer Ⅱ	Silic Beads A +Nuclease-Free Water	Elution Buffer
Volume	200μL + 20 μL + 400 μL	/	800 μL	800 μL	20 μL + 80 μL	30 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） **cfDNA Binding Buffer is removed and added manually after lysis, all other reagents can be added in advance.**

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

2.2 INPUT plasma (200 ~ 600 μL) to the Strip Location 1.

2.3 Place the sample-loaded 96 hole deep hole 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 (°C)
1	1	Lysis	20	0	0	800	5	95

Step 3: Nucleic acid extraction

1. After lysis, open the door of Auto-Pure 32A and Place the 96 hole deep hole plate to 4 ℃ for 5 min.
2. add 400μL cfDNA Binding Buffer into the 1/8st location of the 96 hole deep hole plate.
3. After 96 hole deep hole plate is returned to its original position, run the "cfDNA 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 (°C)
1	4	Beads Transform	0.5	60	0.0	100	5	OFF
2	1	DNA Binding	10.0	360	0.0	6000	7	OFF
3	2	Wash Buffer I	2.0	60	0.0	800	5	OFF
4	3	Wash Buffer II	2.0	60	1.0	800	5	OFF
5	5	Elution	5.0	60	0.0	50	5	37
6	3	Absorb Beads	1.0	0	0.0	800	5	OFF

Step 4: Nucleic acid transform

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

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