

# Extiquick™ Blood/Cell/Tissue DNA Kit

## Operating instruction

(Cat#EX001,Version1.5)

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For Research Use Only.

Not For Use in Diagnostic Procedures.

仅供科研使用

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## Overview

The kit is intended for fast and easy genomic DNA (gDNA) isolation from 50  $\mu$ L - 1 mL of fresh or frozen anticoagulated whole blood, cells, swab, saliva, tissue sample. This kit utilizes homogenised hydroxyl superparamagnetic particle paired with unique reagents for the effective removal of various impurities and specific adsorption of gDNA. The workflow does not require organic reagents such as phenol or chloroform, and highly purified gDNA can be obtained reproducibly within 1 h.

The extracted gDNA can be directly used for downstream experiments, such as enzyme digestion, PCR, qPCR, library preparation, and Southern Blot.

## Component

Product	Component	100 preps (EX001-100)	250 preps (EX001-250)	Storage	Expiry date
Extiquick™ Blood/Cell /Tissue DNA Kit	FLA Buffer	50 mL	125 mL	Room temp	24 months
	Proteinase K	50 mg	150 mg	-20 °C	
	Protease Dissolve Buffer	5 mL	8 mL	2 ~ 8 °C	
	GD Lysis Buffer	45 mL	112.5 mL	Room temp	
	Wash Buffer G I	60 mL	150 mL	Room temp	
	Wash Buffer G 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 °C	

## Storage conditions

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

## Application scope

This product is suitable for whole blood, cells, swab, saliva, tissue sample DNA extraction.

## Self-prepared Material

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

Nuclease-free ddH<sub>2</sub>O and tube

Ethanol (100 %)

1X PBS Buffer

## 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 Wash Buffer G I and Wash Buffer G II according to the table below or 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 °C water bath and mixed well before use.
4. Perform all steps at room temperature (15 ~ 25 °C).
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 °C 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 °C for one year, while Proteinase K solution should be stored at -30 ~ -15 °C 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  $\mu$ L 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  $\mu$ L of 1 $\times$  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  $\mu$ L GD Lysis Buffer, 20  $\mu$ L 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  $^{\circ}$ C, dissolved completely and mixed well before use.

**2.2** Place the sample in a 58  $^{\circ}$ C shaking metal bath for 30 min ~ 3 h. Mix upside down 3 times during the digestion.

**!** **Notes:**

**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  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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  $^{\circ}$ C.

## Automatic Machine operation

### Step 1: Sample Preparation

#### 1.1 Blood sample

1.1.1 Gently mix the blood collection tube upside down ten times, and take 200  $\mu$ L 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 Add 450  $\mu$ L GD Lysis Buffer, 20  $\mu$ L Proteinase K (20 mg/mL) to the sample, vortex and mix well.

1.1.4 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  $\mu$ L of 1 $\times$  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 Add 450  $\mu$ L GD Lysis Buffer, 20  $\mu$ L Proteinase K (20 mg/mL) to the sample, vortex and mix well.

1.2.5 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 Add 450  $\mu$ L GD Lysis Buffer, 20  $\mu$ L 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 $\mu$ L	Wash Buffer G II 800 $\mu$ L	/	Silic Beads B 20 $\mu$ L	Elution Buffer 80 $\mu$ L

**! 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 ( $\mu$ L)	Mix Speed (1-10)	Temp ( $^{\circ}$ C)
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  $\mu$ L 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 ( $\mu$ L)	Mix Speed (1-10)	Temp ( $^{\circ}$ C)
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 ddH <sub>2</sub> O 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. Wash Buffer G I and G II 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.	Wash Buffer G I WA wash has been omitted, or only one wash has been performed. Wash twice with Wash Buffer G I WA in accordance with the Instructions for Use.
	3. Salt ion contamination.	Wash Buffer G II WB wash has been omitted, or Wash Buffer G II 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 $\mu$ l and extend lysis time ( $\geq 15$ min) to ensure complete lysis.
	2. Cell samples.	The input of cells should be less than $1 \times 10^7$ .

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