

Blood / Cultured Cell Genomic DNA Extraction Maxi Kit

Description

The Blood Cell Genomic DNA Extraction Maxi Kit is designed for purification of total DNA (including genomic, mitochondrial and viral DNA) from large volumes of blood sample or cultured cells using a safe protocol that eliminates the use of organic solvents such as phenol and chloroform. By using proteinase K and lysis buffer, the kits effectively lyse cells and degrade protein. Purified DNA with approximately 20-30 Kb of size is suitable for PCR or other enzymatic reactions.

Features

- **High Purity:** DNA is immediately suitable for a variety of applications, including amplification, digestion, PCR etc.
- **High Speed:** Rapid speed for the isolation of genomic DNA from blood, within 60 minutes.
- **Easy Use:** Based on a five-step process, purities genomic DNA without the use of caustic organic compounds.
- **Safe Use :** The kit uses a spin column tube and removes proteins, nucleases in cell.
It is not necessary to treat the sample with harmful organic solvents such as phenol and chloroform.

Applications

- PCR
- Southern Blotting
- Real time PCR
- AFLP
- RFLP

Sampling

Blood, other body fluids and cultured cells. Up to 10ml human whole blood

DNA Size Range

100bp ~20kb
Binding Capacity of Spin Filter
20 ~100 µl of PCR product

Yield

About 50µg - 150µg of total DNA from 5ml of human whole blood; up to 100-300µg of total DNA from 10ml of human whole blood, depends on the sample types and the number of cells in the sample.

Yield of Purification

Sample	Amounts	Yield
Whole Blood	5 ml	50 ~150µg
Whole Blood	10 ml	100 ~300µg

The yield of genomic DNA is variable depending on the sample types.

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Handling Time

About 60 minutes depending upon the sample types.

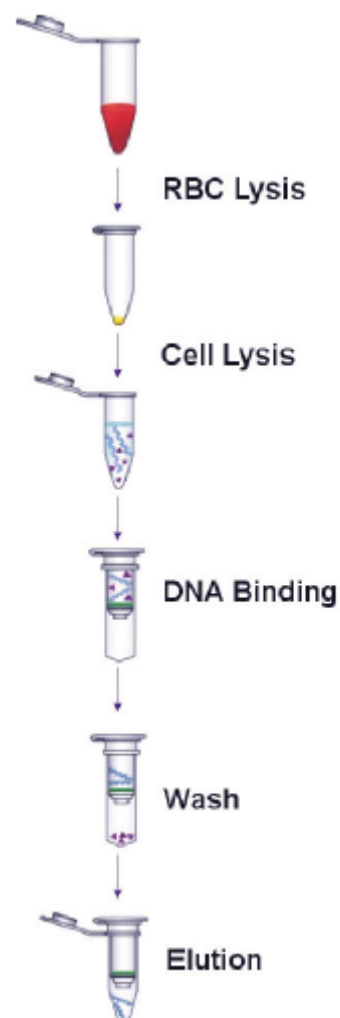
Storage Conditions

Stable for 1 year at room temperature.

The kit is shipped at ambient temperature.

Ordering Information

Cat. No.	Product Name	Size	Kit Components	Store at
DE-003	Blood / Cultured Cell Genomic DNA Extraction Maxi Kit	50 preps.	Proteinase K Powder FSBG Buffer W1 Buffer	Store at room temperature for 1 year. (Except Proteinase K Powder, store at -20°C.)
DE-004		200 preps.	Wash Buffer (conc.) Elution Buffer FSBG Maxi Columns 50 ml Elution tubes	



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Introduction

Genomic DNA Extraction Maxi Kit is an excellent tool offering a speedy and economic method to purify total DNA (e.g. genomic, mitochondrial and viral DNA) from whole blood (fresh or frozen), plasma, serum, buffy coat, body fluids, lymphocytes and cultured cells. This technology first lyses cells and degrades protein by using a chaotropic salt and Proteinase K, then binds DNA to silica-based membranes, washes DNA with ethanol-contained Wash Buffer and then elutes purified DNA by low salt Elution Buffer or ddH₂O. Compare with other harmful and timeconsuming procedures, such as phenol/chloroform extraction and ethanol precipitation, DE-003 and DE-004 shortens the handling time about 1 hour. The size of purified DNA is up to 50 Kb (predominantly 20-30 Kb). After using Genomic DNA Extraction Maxi Kit, the high quality total DNA can be used directly for the downstream applications.

Kit Contents:

	DE-003 (10 preps)	DE-004 (24 preps)
Proteinase K powder	55 mg	130 mg
FSBG Buffer	110 ml	270 ml
W1 Buffer* (concentrated)	33 ml	88 ml
Wash Buffer** (concentrated)	20 ml	40 ml
Elution Buffer	30 ml	60 ml
FSBG Maxi Column	10 pcs	24 pcs
Elution Tube (50 ml tube)	10 pcs	24 pcs
User Manual	1	1

*Add 12 ml / 32 ml of ethanol (96~100%) to W1 Buffer when first open.

**Add 80 ml / 160 ml of ethanol (96~100%) to Wash Buffer when first open.

Specification

Sample Size : • up to 10 ml of fresh/ frozen blood
 • up to 1 x 10⁶ of cultured cells

Column Capacity: up to 500 µg of Genomic DNA

Average DNA yield : 35 µg/ 1 ml whole blood

Format: spin column

Handling Time: about 1 hour

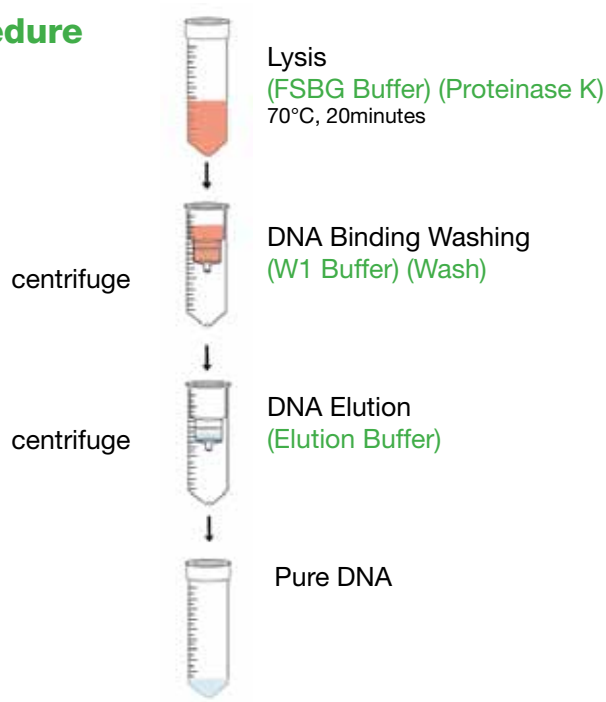
Elution Volume: 1-2 ml

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Important Notes

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. For Cat. No. DE-003, add 5.5 ml of sterile ddH₂O to Proteinase K tube;
For Cat. No. DE-004, add 13 ml of sterile ddH₂O to proteinase K tube to make a 10 mg/ ml stock solution. Vortex and make sure that Proteinase K powder has been completely dissolved. Store the stock solution at 4 °C.
3. For Cat. No. DE-003, add 12 ml of ethanol (96~100 %) to W1 Buffer when first open.
For Cat. No. DE-004, add 32 ml of ethanol (96~100%) to W1 Buffer when first open.
4. For Cat. No. DE-003, add 80 ml of ethanol (96~100%) to Wash Buffer when first open. For Cat. No. DE-004, add 160 ml of ethanol (96~100%) to wash Buffer when first open.
5. Preheat a dry bath or water bath to 70 °C before the operation.
6. Use a centrifuge with a swinging bucket rotor for 15ml (Midi) or 50ml (Maxi) in all centrifugation steps.
The maximum speed should be 3500-5000 rpm or 3000-5000 x g.

Brief Procedure



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• **General Protocol (for Blood DNA Extraction):**

Please Read Important Notes Before Starting The Following Steps.

1. Transfer up to 10 ml sample (whole blood, buffy coat) to a 50 ml centrifuge tube (not provided).
 - **For lymphocytes sample, transfer 10^8 ~ 10^9 cells to a 50 ml centrifuge tube and make total volume to 5 ml with PBS.**
2. Add 500 μ l of Proteinase K (10 mg/ml) to the sample, mix well by vortexing.
And then add 10 ml of FSBG Buffer to the sample mixture.
Mix thoroughly by pulse-vortexing.
 - **Do not add Proteinase K directly to FSBG Buffer.**
3. Incubate the sample mixture in a 70 °C for 20 min to lyse the sample.
During incubation, invert the tube every 3-5 minutes.
 - **At this time, preheat required Elution Buffer or ddH₂O (1~2 ml per sample) to 70 °C. (For DNA Elution step)**
4. (Optional): If RNA-free genomic DNA is required, add 5 μ l of 100 mg/ml RNase A (not provided) to the sample mixture and incubate at room temperature for 10 minutes.
5. Add 8 ml of Isopropanol to the sample mixture. Mix thoroughly by vortexing.
If precipitate appears, break it by pipetting.
6. Place a FSBG Maxi Column to a 50 ml centrifuge tube (not provided).
And transfer 14 ml of sample mixture (ethanol added) (including any precipitate) carefully to the FSBG Maxi Column. Close the cap and centrifuge at 4,000 x g for 5 min.
7. Discard the flow-through and transfer the rest sample mixture to the same FSBG Maxi Column.
Close the cap and centrifuge at 4,000 x g for 5 min and discard the flow-through.
8. Add 4 ml of W1 Buffer (ethanol added) to the FSBG Maxi Column.
Close the cap and centrifuge at 4,000 x g for 5 min.
Discard the flowthrough and place the FSBG Maxi Column back in the 50 ml centrifuge tube.
 - **Make sure that ethanol has been added into W1 Buffer when first open.**
9. Add 7 ml of Wash Buffer (ethanol added) to the FSBG Maxi Column.
Close the cap and centrifuge at 4,000 x g for 5 min.
Discard the flowthrough and place the FSBG Maxi Column back in the 50 ml centrifuge tube.
 - **Make sure that ethanol has been added into W1 Buffer when first open.**
10. Centrifuge at 4,000 x g for an additional 10 min to dry the column.
 - **It might be necessary to dry the column further by placing the column in a vacuum oven at 70 °C for 10 minutes.**
 - **Important Step! The residual liquid can affect the quality of DNA and inhibit subsequent enzymatic reactions.**
11. Place the FSBG Maxi Column into a new 50 ml centrifuge tube. (Elution Tube) (provided)
12. Add 1 ml of preheat Elution Buffer or ddH₂O (pH 7.5- 9.0) to the membrane center of the FSBG Maxi Column. Stand the FSBG Maxi Column for 5 min at room temperature.
 - **Important Step! For effective elution, stand the FSBG Maxi Column for 5 minutes is required to make sure that Elution Buffer is absorbed completely by column membrane.**
 - **Standard volume for elution is 1 ml.**
If higher DNA yield is required, repeat the DNA Elution step (step 12) to increase DNA recovery.
13. Centrifuge at 4,000 x g for 2 minutes to elute total DNA.

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Protocol: (for Cultured Cell DNA Extraction)

Please Read Important Notes Before Starting The Following Steps.

1. Transfer up to 1×10^6 of cells to a 50 ml centrifuge tube (not provided).
Centrifuge at $4,000 \times g$ for 5 minutes to pellet the cells.
 - **If using adherent cells, trypsinize the cells before harvesting.**
2. Resuspend the cells with 10 ml of PBS.
3. Add 500 μ l of Proteinase K (10 mg/ml) to the sample, mix well by vortexing.
4. Add 10 ml of FSBG Buffer to the sample mixture. Mix thoroughly by pulse-vortexing.
 - **Do not add Proteinase K directly to FSBG Buffer.**
5. Incubate the sample mixture at 70 °C for 20 minutes to lyse the sample.
During incubation, invert the tube every 3-5 minutes.
 - **At this time, preheat required Elution Buffer or ddH₂O (1ml per sample) to 70 °C. (For DNA Elution)**
6. Follow the Blood protocol starting from step 4.

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Troubleshooting:

LOW YIELD

1. Too many cells were used
 - **reduce the sample volume.**
2. Poor cell lysis because of insufficient Proteinase K activity
 - **Use a fresh or well-stored Proteinase K stock solution.**
3. Poor cell lysis because of insufficient mixing with FSBG buffer
 - **Mix the sample and FSBG Buffer immediately and thoroughly by pulse-vortexing.**
4. Poor cell lysis because of insufficient incubation time
 - **Extend the incubation time and make sure that no residual particulates remain.**
5. Ethanol is not added into Wash Buffer when first open; the volume or the percentage of ethanol is not correct before adding into Wash Buffer
6. Elution of genomic DNA is not efficient
 - **Make sure the pH of ddH₂O is between 7.5- 8.5.**
 - **After Elution Buffer or ddH₂O is added, stand the FSBG Maxi Column for 5-10 min before centrifugation.**

COLUMN IS CLOGGED

1. Blood sample contains clots
 - **Mix the blood sample well with anti-coagulant to prevent formation of blood clots.**
2. Sample is too viscous
 - **Reduce the sample volume.**

PURIFIED DNA DOSE NOT PERFORM WELL IN DOWNSTREAM APPLICATION

1. Sample is old
 - **Always use fresh or well-stored sample for genomic DNA extraction.**
2. Residual ethanol contamination
 - **After Wash step, centrifuge at 4,000 x g for an additional 10 minutes to dry the FSBG Maxi Column**
3. RNA contamination

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