Genomic DNA Maxi Kit (Blood/Cultured Cell)

For research use only

Sample : 10 ml of frozen blood, cultured cells (up to 1 x 10^8)

Yield : up to 140 µg

Format : spin column

Operation time : within 60 minutes

Elution volume : 1-2 ml

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Introduction

The Genomic DNA Maxi Kit (Blood/Cultured Cell) provides an efficient method for purifying total DNA (including genomic, mitochondrial and viral DNA) from up to 10 ml of frozen blood and cultured cells. Proteinase K is used to reduce hemoglobin contamination while chaotropic salt and detergents are used to lyse cells and degrade protein, allowing the DNA to bind to the glass fiber matrix of the spin column (1). Contaminants are removed using a Wash Buffer (containing ethanol) and the purified genomic DNA is eluted by a low salt Elution Buffer or TE. The entire procedure can be completed in 1 hour without phenol/chloroform extraction or alcohol precipitation. Purified DNA, with approximately 20-30 Kb, is suitable for use in PCR or other enzymatic reactions.

Quality Control

The quality of the Genomic DNA Maxi Kit (Blood/Cultured Cell) is tested on a lot-to-lot basis by isolating genomic DNA from 10 ml of frozen whole human blood. The purified DNA (> 80 µg with an A260/A280 ratio of 1.6 - 1.8) is quantified with a spectrophotometer and checked by electrophoresis.

Kit Contents

Name	GDM02	GDM10	GDM25
GB Buffer	25 ml	120 ml	280 ml
W1 Buffer	10 ml	45 ml	130 ml
Wash Buffer* (Add Ethanol)	5 ml (20 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC)	6 ml	30 ml	60 ml
Proteinase K** (Add ddH_2O)	11 mg (1.1 ml)	55 mg (5.5 ml)	135 mg (13.5 ml)
GD Maxi Column	2 pcs	10 pcs	25 pcs

Order Information

Product Name	Package Size	Cat. No.
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	GB100/300
Genomic DNA Midi Kit (Blood/Cultured Cell)	25 preps	GDI25
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	GDM10/25
Genomic DNA Mini Kit (Tissue)	50/100/300 preps	GT050/100/300
Genomic DNA Mini Kit (Plant)	100 preps	GP100
Genomic DNA Maxi Kit (Plant)	10/25 preps	GPM10/25
96-Well Genomic DNA Kit	2/4/10 x 96 Wells	GBP02/04/10
96-Well Genomic DNA Kit (Plant)	2/4/10 x 96 Wells	GPP02/04/10
Vacuum Manifold (Accessories)	1 set	ZFV04

*Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume)

**Add ddH_2O to prepare Proteinase K (vortex to dissolve and spin down) and store at 4ºC (see the bottle label for volume)

Caution

GB Buffer and W1 Buffer contain guanidine hydrochloride which is a harmful irritant. During operation, always wear a lab coat, disposable gloves, and protective goggles.

References

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615

Genomic DNA Maxi Kit (Blood/Cultured Cell) Frozen Blood Protocol

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Add ddH_20 (see the bottle label for volume) to prepare Proteinase K (vortex to dissolve and spin down) and store at 4ºC
- Additional requirements: centrifuge tubes, absolute ethanol, RNase A (10 mg/ml)

Step	Procedure
Step 1 Cell Lysis	• Add **500 µl of Proteinase K** and up to 10 ml of frozen blood to a 50 ml centrifuge tube and mix by shaking briefly. • Incubate the mixture at 60°C for 15 minutes. During incubation, invert the tube every 3 minutes. • Add **10 ml of GB Buffer** to the 50 ml centrifuge tube and mix by shaking vigorously. • Incubate the mixture in a 70°C water bath for 20 minutes or until the sample lysate is clear. During incubation, invert the tube every 3-5 minutes. • At this time, preheat the required **Elution Buffer** (2 ml per sample) in a 70°C water bath (for Step 4 DNA Elution). **Optional Step: RNA Degradation** If RNA-free genomic DNA is required, perform this optional step. • Add 50 µl of RNase A (10 mg/ml) to the sample lysate and mix by shaking vigorously. • Incubate at room temperature for 10 minutes.
Step 2 DNA Binding	• Add 10 ml of absolute ethanol to the sample lysate and immediately mix by shaking vigorously for 10 seconds. • Place a **GD Maxi Column** in a 50 ml centrifuge tube. • Transfer all of the mixture (including any precipitate) to the **GD Maxi Column**. • Centrifuge at 4,000 x g for 10 minutes.
Step 3 Wash	• Add **4 ml of W1 Buffer** into the **GD Maxi Column**. • Centrifuge at 4,000 x g for 3 minutes. • Discard the flow-through and place the **GD Maxi Column** back in the 50 ml centrifuge tube. • Add **6 ml of Wash Buffer** (ethanol added) to the **GD Maxi Column**. • Centrifuge at 4,000 x g for 3 minutes to wash again. • Discard the flow-through and place the **GD Maxi Column** back in the 50 ml centrifuge tube. • Centrifuge at 4,000 x g for 10 minutes to dry the column matrix.
Step 4 DNA Elution	Standard elution volume is 1 ml. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 2 ml. • Transfer the dried **GD Maxi Column** to a clean 50 ml centrifuge tube. • Add **1 ml of preheated Elution Buffer** or TE into the center of the column matrix. • Incubate at 60ºC for 3 minutes. • Centrifuge at 4,000 x g for 5 minutes at room temperature to elute the purified DNA.

Genomic DNA Maxi Kit (Blood/Cultured Cell) Cultured Cell Protocol

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Add ddH_20 (see the bottle label for volume) to prepare Proteinase K (vortex to dissolve and spin down) and store at 4ºC
- Additional requirements: PBS (phosphate-buffered saline), centrifuge tubes, absolute ethanol, RNase A (10 mg/ml)

Step	Procedure
Step 1 Cell Lysis	**If using adherent cells, trypsinize the cells before harvesting**. • Transfer cells (up to 1 x 10^8) to a 50 ml centrifuge tube and harvest with centrifugation for 5 minutes at 4,000 x g. • Resuspend the cells with 10 ml of PBS. • Add **500 µl of Proteinase K** to a 50 ml centrifuge tube and mix by shaking briefly. • Add **10 ml of GB Buffer** to the 50 ml centrifuge tube and mix by shaking vigorously. • Incubate the mixture in a 70ºC water bath for 20 minutes or until the sample lysate is clear. During incubation, invert the tube every 3-5 minutes. • At this time, preheat the required **Elution Buffer** (2 ml per sample) in a 70ºC water bath (for Step 4 DNA Elution). **Optional Step: RNA Degradation** If RNA-free genomic DNA is required, perform this optional step. • Add 50 µl of RNase A (10 mg/ml) to the sample lysate and mix by shaking vigorously. • Incubate at room temperature for 10 minutes.
Step 2 DNA Binding	• Add 10 ml of absolute ethanol to the sample lysate and immediately mix by shaking vigorously for 10 seconds. If precipitate appears, break it up by pipetting. • Place a **GD Maxi Column** in a 50 ml centrifuge tube. • Add 15 ml of the mixture (including any precipitate) to the **GD Maxi Column**. • Centrifuge at 4,000 x g for 5 minutes. • Discard the flow-through and place the **GD Maxi Column** back in the 50 ml centrifuge tube. • Transfer the remaining mixture to the **GD Maxi Column**. • Centrifuge at 4,000 x g for 5 minutes. • Discard the flow-through and place the **GD Maxi Column** back in the 50 ml centrifuge tube.
Step 3 Wash	• Add **4 ml of W1 Buffer** into the **GD Maxi Column**. • Centrifuge at 4,000 x g for 3 minutes. • Discard the flow-through and place the **GD Maxi Column** back in the 50 ml centrifuge tube. • Add **6 ml of Wash Buffer** (ethanol added) to the **GD Maxi Column**. • Centrifuge at 4,000 x g for 3 minutes to wash again. • Discard the flow-through and place the **GD Maxi Column** back in the 50 ml centrifuge tube. • Centrifuge at 4,000 x g for 10 minutes to dry the column matrix.
Step 4 DNA Elution	Standard elution volume is 1 ml. If higher DNA yield is required, repeat the DNA Elution Step to increase DNA recovery and the total elution volume to approximately 2 ml. • Transfer the dried **GD Maxi Column** to a clean 50 ml centrifuge tube. • Add **1 ml of preheated Elution Buffer** or TE into the center of the column matrix. • Incubate at 60ºC for 3 minutes. • Centrifuge at 4,000 x g for 5 minutes at room temperature to elute the purified DNA.

Troubleshooting

Problem	Possible Reasons/Solution
Clogged Column	**Too much sample was used** • Reduce the sample volume or separate it into multiple tubes. **Precipitate was formed at DNA Binding Step** • Reduce the sample material. • Prior to loading the column, break up precipitate in the ethanol-added lysate.
Low Yield	**Incorrect DNA Elution Step** • Ensure that the Elution Buffer or TE is added to the center of the GD Maxi Column matrix and is absorbed completely. • Ensure to preheat the Elution Buffer or TE prior to adding to the GD Maxi Column. **Incomplete DNA Elution** • Elute twice to increase yield.
Eluted DNA does not perform well in downstream applications	**Residual ethanol contamination** • Following the wash step, dry the GD Maxi Column with additional centrifugation at full speed for 10 minutes or incubate at 60ºC for 10 minutes. **RNA contamination** • Perform the optional RNA Degradation Step.