Geneaid

Instruction Manual
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For Research Use Only

Genomic DNA Maxi Kit (Blood/Cultured Cell)

GDM002 (2 Preparation Sample Kit)
GDM010 (10 Preparation Kit)
GDM025 (25 Preparation Kit)

Advantages

Sample: up to 10 ml of whole blood (fresh or frozen), up to 1 x 10^8 cultured cells
Yield: up to 200 µg of genomic DNA from 10 ml of whole blood
Format: genomic DNA maxi columns
Time: within 60 minutes
Elution Volume: 500 µl - 2 ml
Kit Storage: dry at room temperature (15-25°C)

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Geneaid Biotech Ltd.
Tel: 886 2 26960999 · Fax: 886 2 26960599 · www.geneaid.com · info@geneaid.com

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 fresh or frozen blood and cultured cells. Protease 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. Contaminants are removed using a Wash Buffer (containing ethanol) and the purified genomic DNA is eluted by a low salt Elution Buffer, TE or water. The entire procedure can be completed within 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 purifying genomic DNA from 10 ml of whole human blood. The purified DNA is quantified with a spectrophotometer and analyzed by electrophoresis.

Kit Components

Component	GDM002	GDM010	GDM025
GB Buffer	25 ml	120 ml	280 ml
Protease[1]	1.1 ml	5.5 ml	6.5 ml x 2
W1 Buffer	10 ml	45 ml	130 ml
Wash Buffer[2] (Add Ethanol)	5 ml (20 ml)	25 ml (100 ml)	25 ml x 1 (100 ml) 50 ml x 1 (200 ml x 1)
Elution Buffer	6 ml	30 ml	60 ml
GD Maxi Columns in Collection Tube	2	10	25
Collection Tube with Cap	2	10	25

[1]Protease is shipped at room temperature and should be stored at 2-8ºC for up to 6 months.

[2]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

Quick Protocol Diagram

Transfer up to 10 ml of whole blood or up to 1 x 10^8 cultured cells to a 50 ml centrifuge tube

Lysis

DNA binding to membrane while contaminants remain suspended

Wash (removal of contaminants while DNA remains bound to membrane)

Elution of pure genomic DNA which is ready for subsequent reactions

Genomic DNA Maxi Kit (Blood/Cultured Cell) Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.

IMPORTANT BEFORE USE!

1. Protease is shipped at room temperature and should be stored at 2-8ºC for up to 6 months.

2. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Additional Requirements

phosphate-buffered saline (PBS), centrifuge tubes, absolute ethanol, RNase A (10 mg/ml), 60ºC incubator or water bath.

Protocol Procedure

1. Sample Preparation

Fresh or Frozen Blood

Transfer **500 µl of Protease** to the bottom of a 50 ml centrifuge tube. Add **3-10 ml of whole blood**.

Note: If the blood volume is less than 10 ml, add the appropriate volume of PBS.

Adherent Cultured Animal Cells (trypsinize cells prior to harvesting)

Remove the culture medium and wash cells in PBS. Aspirate PBS and add **0.10-0.25% Trypsin in PBS**. Once cells detach add medium then transfer to a 50 ml centrifuge tube. Proceed with Suspension Cultured Animal cells.

Suspension Cultured Animal Cells

Transfer **cells (up to 1 x 10^8)** to a 50 ml microcentrifuge tube then centrifuge for 5 minutes at 300 x g. Discard the supernatant then resuspend cells in **10 ml of PBS**. Add **500 µl of Protease** into the 50 ml centrifuge tube then mix by shaking briefly.

2. Lysis

Add **10 ml of GB Buffer** into the centrifuge tube and mix the sample thoroughly by inverting the tube 10 times and followed by vigorous shaking.

NOTE: It is essential that the sample and GB Buffer are mixed thoroughly to yield a homogenous solution. DO NOT add protease directly to GB Buffer before use.

Incubate the sample mixture at 60ºC for 20 minutes. During incubation, invert the tube every 5 minutes. At this time, pre-heat the required volume of Elution Buffer (2 ml/ sample) to 60ºC for step 5 DNA elution.

Optional Step: RNA Degradation

Following 60ºC incubation, add 50 µl of RNase A (10 mg/ml) to the clear lysate and shake vigorously. Incubate at room temperature for 10 minutes.

3. DNA Binding

Add **10 ml of absolute ethanol to the sample lysate** and vortex immediately for 10 seconds.

NOTE: If precipitate appears, break it up as much as possible with a pipette.

Transfer **15 ml of the sample mixture** (including any precipitate) to the **GD Maxi Column in Collection Tube**. Close the cap and centrifuge at 3,000 x g for 3 minutes then discard the flow-through. Place the **GD Maxi Column** back in the **Collection Tube** then transfer the remaining mixture to the **GD Maxi Column**. Close the cap and centrifuge at 3,000 x g for 3 minutes then discard the flow-through.

4. Wash

Place the **GD Maxi Column** back in the **Collection Tube** and add **4 ml of W1 Buffer** into the **GD Maxi Column**. Close the cap and centrifuge at 3,000 x g for 2 minutes then discard the flow-through. Place the **GD Maxi Column** back in the **Collection Tube**. Add **12 ml of Wash Buffer (make sure ethanol was added)** to the **GD Maxi Column** then let stand for 2 minutes. Close the cap and centrifuge at 3,000 x g for 2 minutes then discard the flow-through. Place the **GD Maxi Column** back in the **Collection Tube** then centrifuge at 3,000 x g for 10 minutes to dry the column matrix

5. Elution

Transfer the dried **GD Maxi Column** to a new **Collection Tube**. Add **500 µl-1 ml of pre-heated Elution Buffer**[1], TE[2] or water[3] into the **CENTER** of the column matrix. Incubate at room temperature for 3 minutes then centrifuge at 3,000 x g for 5 minutes to elute the purified DNA. For maximum DNA concentration: Reload the eluate containing the DNA into the center of the column matrix. Incubate at room temperature for 3 minutes then centrifuge at 3,000 x g for 5 minutes to elute the purified DNA again. For maximum DNA yield: Repeat the elution step by adding 500 µl-1 ml of pre-heated Elution Buffer into the center of the column matrix again. Incubate at room temperature for 3 minutes then centrifuge at 3,000 x g for 5 minutes to elute the purified DNA. The total elution volume is approximately 1-2 ml.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the CENTER of the column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the CENTER of the column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the CENTER of the column matrix and is completely absorbed. DNA eluted in water should be stored at -20ºC to avoid degradation.

Troubleshooting

Low Yield

Incomplete buffer preparation

Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

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.

Incorrect DNA elution step.

Ensure that Elution Buffer, TE or water is added into the **CENTER** of the GD Maxi Column matrix and is completely absorbed. Use pre-heated Elution Buffer, TE, or water (60~70ºC). If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Repeating the elution step will increase yield. Repeating the elution step using the eluate only will increase DNA concentration.

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 3,000 x g for 5 minutes to ensure the membrane is completely dry.

Genomic DNA Maxi Kit (Blood/Cultured Cell) Functional Test Data

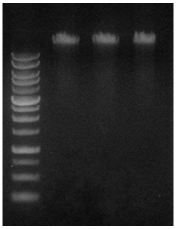

Figure 1. Genomic DNA from 10 ml whole blood samples was extracted using the Genomic DNA Maxi Kit (Blood/Cultured Cell). 0.5 µl aliquots from 1 ml eluates of purified genomic DNA were analyzed by electrophoresis on a 0.8% agarose gel. M = Geneaid 1 Kb DNA Ladder

Sample	DNA Conc.	260/280	260/230	Yield
1	180.6 ng/µl	1.84	2.28	180.6 µg
2	195.8 ng/µl	1.86	2.15	195.8 µg
3	187.5 ng/µl	1.84	2.21	187.5 µg

Related DNA Extraction Products

Genomic DNA Extraction		
Product	Package Size	Catalogue Number
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	GB100/300
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	GDM010/25
Genomic DNA Mini Kit (Tissue)	50/100/300 preps	GT050/100/300
gSYNC™ DNA Extraction Kit	50/100/300 preps	GS050/100/300
Genomic DNA Mini Kit (Plant)	100 preps	GP100
Genomic DNA Maxi Kit (Plant)	10/25 preps	GPM10/25
GENEzol™ DNA Reagent Plant	100/200 rxns	GR100/200
Presto™ Mini gDNA Yeast Kit	100/300 preps	GBY100/300
Presto™ Mini gDNA Bacteria Kit	100/300 preps	GBB100/101/300/301
Geneius™ Micro DNA Extraction Kit	100/300 preps	GMB100/300
Presto™ Buccal Swab gDNA Extraction Kit	100/300 preps	GSK100/300
Presto™ 96 Well Blood Genomic DNA Extraction Kit	4/10 x 96 preps	96GBP04/10
Presto™ 96 Well Plant Genomic DNA Extraction Kit	4/10 x 96 preps	96GPP04/10
DNA RNA Purification		
Product	Package Size	Catalogue Number
Presto™ DNA/RNA Extraction Kit	50/100 preps	DR050/100
Presto™ DNA/RNA/Protein Extraction Kit	50/100 preps	DRP050/100

For additional product information please visit www.geneaid.com. Thank you!

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