Genomic DNA Mini Kit (Blood/Cultured Cell)

For research use only

Sample: up to 300 µl of whole fresh blood, up to 1 x 10^7 cultured animal cells
up to 200 µl of buffy coat, up to 2 x 10^8 yeast/fungus

Format: RBC Lysis Buffer and spin column purification

Yield: 4-6 µg from 200 µl of whole blood

Time: within 25 minutes

Elution volume: 30-200 µl

Storage: dry at room temperature (15-25ºC) for up to 1 year

CERTIFICATE NO. QAIC/TW/50077

ISO 9001:2008 QMS

Introduction

The Genomic DNA Mini Kit (Blood/Cultured Cell) provides an efficient method for purifying total DNA (including genomic, mitochondrial and viral DNA) from whole fresh blood, cultured animal cells, buffy coat, yeast and other fungus species. Chaotropic salt is used to lyse cells and degrade protein, allowing 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, TE or water. The entire procedure can be completed within 25 minutes without phenol/chloroform extraction or alcohol precipitation. The 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 Mini Kit (Blood/Cultured Cell) is tested on a lot-to-lot basis by isolating genomic DNA from 200 µl of whole fresh human blood. The purified DNA (4-6 µg with an A260/A280 ratio of 1.6 - 1.8) is quantified with a spectrophotometer and analyzed by electrophoresis.

Kit Contents

Component	GB004	GB100	GB300
RBC Lysis Buffer	6 ml	135 ml	405 ml
GT Buffer	1.5 ml	30 ml	75 ml
GB Buffer	2 ml	40 ml	100 ml
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer* (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer	1 ml	30 ml	75 ml
GD Column	4 pcs	100 pcs	300 pcs
2 ml Collection Tube	8 pcs	200 pcs	600 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
Geneius™ Micro gDNA Extraction Kit	100/300 preps	GMB100/300
Presto™ Buccal Swab gDNA Extraction Kit	100/300 preps	GSK100/300
Presto™ Mini gDNA Yeast Kit	100/300 preps	GBY100/300
Presto™ Mini gDNA Bacteria Kit	100/300 preps	GBB100/300
gSYNC™ DNA Extraction Kit	50/100/300 preps	GS050/100/300
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

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

Caution

GB Buffer contains guanidine hydrochloride. 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

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

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Collect blood in EDTA-NA_2 treated tubes (or other anticoagulant mixtures)

Additional requirements: microcentrifuge tubes, centrifuge tube, absolute ethanol, (optional) RNase A (10 mg/ml)

Sample Preparation	**Fresh Blood** • Transfer **up to 300 µl of blood** to a 1.5 ml microcentrifuge tube. NOTE: If the blood sample is more than 300 µl (up to 1 ml), add to a sterile 15 ml centrifuge tube. • Add **3X the sample volume of RBC Lysis Buffer** then mix by inversion. Do not vortex. • Incubate the tube for 10 minutes at room temperature. • Centrifuge for 5 minutes at 3,000 x g then remove the supernatant completely. • Add **100 µl of RBC Lysis Buffer** to resuspend the leukocyte pellet then proceed with Cell Lysis.
Step 1 Cell Lysis	• Add **200 µl of GB Buffer** to the 1.5 ml microcentrifuge tube then shake vigorously. • Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear. • During incubation, invert the tube every 3 minutes. At this time, preheat the required **Elution Buffer** (200 µl per sample) to 60ºC (for Step 4 DNA Elution). **Optional Step: RNA Degradation** (If RNA-free gDNA is required, perform this optional step) • Following 60ºC incubation, add 5 µl of RNase A (10 mg/ml) to the clear lysate then mix by shaking vigorously. • Incubate at room temperature for 5 minutes.
Step 2 DNA Binding	• Add **200 µl of absolute ethanol** to the lysate then immediately mix by shaking vigorously for 10 seconds. NOTE: If precipitate appears, break it up as much as possible with a pipette. • Place a **GD Column** in a **2 ml Collection Tube**. • Transfer the mixture (including any precipitate) to the **GD Column**. • Centrifuge at 14-16,000 x g for 5 minutes. • Discard the **2 ml Collection Tube** then place the **GD Column** in a new **2 ml Collection Tube**.
Step 3 Wash	• Add **400 µl of W1 Buffer** to the **GD Column** then centrifuge at 14-16,000 x g for 30-60 seconds. • Discard the flow-through then place th**e GD Column** back in th**e 2 ml Collection Tube**. • Add **600 µl of Wash Buffer** (make sure ethanol was added) to the **GD Column**. • Centrifuge at 14-16,000 x g for 30-60 seconds then discard the flow-through. • Place the **GD Column** back in the **2 ml Collection Tube**. • Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.
Step 4 DNA Elution	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl. • Transfer the dried **GD Column** to a clean 1.5 ml microcentrifuge tube. • Add **100 µl of pre-heated Elution Buffer** or TE to the center of the column matrix. • Let stand for at least 3 minutes to ensure the **Elution Buffer** or TE is completely absorbed. • Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.

Genomic DNA Mini Kit (Blood/Cultured Cell) Buffy Coat Protocol

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

Additional requirements: microcentrifuge tubes, centrifuge tube, absolute ethanol, (optional) RNase A (10 mg/ml)

Sample Preparation	**Buffy Coat** • Transfer **up to 200 µl of buffy coat** to a 1.5 ml microcentrifuge tube. • Add **3X the sample volume of RBC Lysis Buffer** to the tube then mix by inversion. • Incubate the tube for 10 minutes at room temperature. During incubation, invert the tube every 3 minutes. • Centrifuge at 14-16,000 x g for 1 minute then discard the supernatant completely. • Add **500 µl of RBC Lysis Buffer** to resuspend the leukocyte pellet. • Centrifuge at 14-16,000 x g for 1 minute then discard the supernatant completely. • Add **200 µl of RBC Lysis Buffer** to the tube then resuspend the leukocyte pellet completely. NOTE: Mix the tube by vortex only if the pellet is not resuspended completely and the column becomes barred.
Step 1 Cell Lysis	• Add **250 µl of GB Buffer** to the 1.5 ml microcentrifuge tube then shake vigorously. • Incubate at 60ºC for at least 30 minutes to ensure the sample lysate is clear. • During incubation, invert the tube every 3 minutes. At this time, preheat the required **Elution Buffer** (200 µl per sample) to 60ºC (for Step 4 DNA Elution). **Optional Step: RNA Degradation** (If RNA-free gDNA is required, perform this optional step) • Following 60ºC incubation, add 5 µl of RNase A (10 mg/ml) to the clear lysate then mix by shaking vigorously. • Incubate at room temperature for 5 minutes.
Step 2 DNA Binding	• Add **250 µl of absolute ethanol** to the lysate then immediately mix by shaking vigorously for 10 seconds. NOTE: If precipitate appears, break it up as much as possible with a pipette. • Place a **GD Column** in a **2 ml Collection Tube**. • Transfer the mixture (including any precipitate) to the **GD Column**. • Centrifuge 14-16,000 x g for 5 minutes. • Discard the **2 ml Collection Tube** then place the **GD Column** in a new **2 ml Collection Tube**.

Step 3 Wash	• Add **400 µl of W1 Buffer** to the **GD Column** then centrifuge at 14-16,000 x g for 30-60 seconds. • Discard the flow-through then place th**e GD Column** back in th**e 2 ml Collection Tube**. • Add **600 µl of Wash Buffer** (make sure ethanol was added) to the **GD Column**. • Centrifuge at 14-16,000 x g for 30-60 seconds then discard the flow-through. • Place the **GD Column** back in the **2 ml Collection Tube**. • Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.
Step 4 DNA Elution	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl. • Transfer the dried **GD Column** to a clean 1.5 ml microcentrifuge tube. • Add **100 µl of pre-heated Elution Buffer** or TE to the center of the column matrix. • Let stand for at least 10 minutes to ensure the **Elution Buffer** or TE is completely absorbed. • Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.

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

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

Additional requirements: microcentrifuge tubes, centrifuge tube, absolute ethanol, phosphate-buffered saline (PBS), 0.10-0.25% Trypsin, (optional) RNase A (10 mg/ml)

Sample Preparation	**Adherent Cultured Animal Cells** (trypsinize cells prior to harvesting) 1. Remove the culture medium and wash cells in PBS. Aspirate PBS and add 0.10-0.25% Trypsin in PBS. 2. Once cells detach add medium then transfer to a 15 ml centrifuge tube. 3. Proceed with Suspension Cultured Animal cells. **Suspension Cultured Animal Cells** • **Transfer cells (up to 1 x 10^7)** to a 1.5 ml microcentrifuge tube then centrifuge for 5 minutes at 300 x g. • Discard the supernatant then resuspend cells in **150 µl of RBC Lysis Buffer** by pipette. Proceed with Step 1. **Up to 50 µl of non-nucleated mammalian blood or up to 10 µl of nucleated erythrocytes (e.g. bird or fish)** • Add **150 µl of GT Buffer** and blood sample to a 1.5 ml microcentrifuge tube then shake vigorously.
Step 1 Cell Lysis	• Add **200 µl of GB Buffer** to the 1.5 ml microcentrifuge tube then shake vigorously. • Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear. • During incubation, invert the tube every 3 minutes. At this time, preheat the required **Elution Buffer** (200 µl per sample) to 60ºC (for Step 4 DNA Elution). **Optional Step: RNA Degradation** (If RNA-free gDNA is required, perform this optional step) • Following 60ºC incubation, add 5 µl of RNase A (10 mg/ml) to the clear lysate then mix by shaking vigorously. • Incubate at room temperature for 5 minutes.
Step 2 DNA Binding	• Add **200 µl of absolute ethanol** to the lysate then immediately mix by shaking vigorously for 10 seconds. NOTE: If precipitate appears, break it up as much as possible with a pipette. • Place a **GD Column** in a **2 ml Collection Tube**. • Transfer the mixture (including any precipitate) to the **GD Column**. • Centrifuge at 14-16,000 x g for 2 minutes. • Discard the **2 ml Collection Tube** then place the **GD Column** in a new **2 ml Collection Tube**.
Step 3 Wash	• Add **400 µl of W1 Buffer** to the **GD Column** then centrifuge at 14-16,000 x g for 30-60 seconds. • Discard the flow-through then place th**e GD Column** back in th**e 2 ml Collection Tube**. • Add **600 µl of Wash Buffer** (make sure ethanol was added) to the **GD Column**. • Centrifuge at 14-16,000 x g for 30-60 seconds then discard the flow-through. • Place the **GD Column** back in the **2 ml Collection Tube**. • Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.
Step 4 DNA Elution	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl. • Transfer the dried **GD Column** to a clean 1.5 ml microcentrifuge tube. • Add **100 µl of pre-heated Elution Buffer** or TE to the center of the column matrix. • Let stand for at least 3 minutes to ensure the **Elution Buffer** or TE is completely absorbed. • Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.

Genomic DNA Mini Kit (Blood/Cultured Cell) Yeast/Fungus Protocol

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

Additional requirements: microcentrifuge tubes, absolute ethanol, lyticase or zymolase, 50 mM EDTA pH8.0, (optional) RNase A (50 mg/ml)

Cell Harvesting Pre-Lysis	**Yeast/Fungus** • Transfer **yeast/fungus cells (up to 2 x 10^8)** to a 1.5 ml microcentrifuge tube. • Harvest cells by centrifugation for 10 minutes at 5,000 x g. • Discard the supernatant and resuspend the pellet in **600 µl of 50 mM EDTA pH8.0**. • Add **200 U of lyticase or zymolase** then incubate at 30ºC for 30 minutes. • Centrifuge the mixture for 10 minutes at 2,000 x g to harvest the spheroplast. • Remove the supernatant then add **200 µl of GT Buffer**. • Resuspend the cell pellet by shaking vigorously or pipetting then incubate at room temperature for 5 minutes.
Step 1 Cell Lysis	• Add **200 µl of GB Buffer** to the sample and mix by shaking vigorously for 5 seconds. • Incubate at 60ºC for 10 minutes to ensure the sample lysate is clear. NOTE: During incubation, invert the tube every 3 minutes. At this time, preheat the required **Elution Buffer** (200 µl per sample) to 60ºC (for Step 4 DNA Elution). **Optional Step: RNA Degradation** (If RNA-free gDNA is required, perform this optional step) • Following 60ºC incubation, add 5 µl of RNase A (50 mg/ml) to the clear lysate then mix by shaking vigorously. • Incubate at room temperature for 10 minutes.
Step 2 DNA Binding	• Add **200 µl of absolute ethanol** to the sample lysate then immediately mix by shaking vigorously. NOTE: If precipitate appears, break it up as much as possible with a pipette. • Place a **GD Column** in a **2 ml Collection Tube**. • Transfer the mixture (including any precipitate) to the **GD Column**. • Centrifuge at 14-16,000 x g for 2 minutes. • Discard the **2 ml Collection Tube** containing the flow-through. • Place the **GD Column** in a new **2 ml Collection Tube**.
Step 3 Wash	• Add **400 µl of W1 Buffer** to the **GD Column** then centrifuge at 14-16,000 x g for 30 seconds • Discard the flow-through then place th**e GD Column** back in the **2 ml Collection Tube**. • Add **600 µl of Wash Buffer** (make sure ethanol was added) to the **GD Column**. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through then place the **GD Column** back in the **2 ml Collection Tube**. • Centrifuge for 3 minutes at 14-16,000 x g to dry the column matrix.
Step 4 DNA Elution	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl. • Transfer the dried **GD Column** to a clean 1.5 ml microcentrifuge tube. • Add **100 µl of pre-heated Elution Buffer** or TE to the center of the column matrix. • Let stand for at least 3 minutes to ensure the **Elution Buffer** or TE is completely absorbed. • Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.

Troubleshooting

Problem	Possible Reasons/Solution
Clogged Column	• Reduce sample volume or separate into multiple tubes.
Low Yield	**Precipitate was formed at DNA Binding step** • Reduce the sample material. • Following ethanol addition, break up any precipitate as much as possible prior to loading GD Column. • Ensure that Elution Buffer or TE is added to the center of the GD Column and is absorbed completely. • Elute twice to increase yield.
Eluted DNA does not perform well in downstream applications	• Following the Wash Step, dry the GD Column by centrifuge at 14-16,000 x g for 5 minutes or incubate at 60ºC for 5 minutes. • Use fresh blood as long term storage may result in fragmentation of genomic DNA.