

Genomic DNA Mini Kit (Blood/Cultured Cell)

For research use only

- Sample** : up to 300 µl of whole blood, up to 200 µl of frozen blood, up to 200 µl of buffy coat, cultured animal cells (up to 1×10^7), cultured bacterial cells (up to 1×10^9) and fungus cells (up to 5×10^7)
- Operation** : centrifuge
- Format** : spin column
- Yield** : up to 50 µg
- Operation time** : within 60 minutes
- Elution volume** : 50-200 µl

Geneaid



www.geneaid.com

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 blood, frozen blood, buffy coat, cultured animal/bacterial cells and fungus. 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 or TE. The entire procedure can be completed in 1 hour without phenol/chloroform extraction or alcohol precipitation, with an average DNA yield of 6 µg from 200 µl of whole human blood and up to 50 µg of DNA from 200 µl of buffy coat. 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 human blood. The purified DNA (4-6 µg with an A260/A280 ratio of 1.6 - 1.8) is quantified with a spectrophotometer and checked by electrophoresis.

Kit Contents

Name	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
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 (see the bottle label for volume) to the Wash Buffer prior to initial use

Caution

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

References

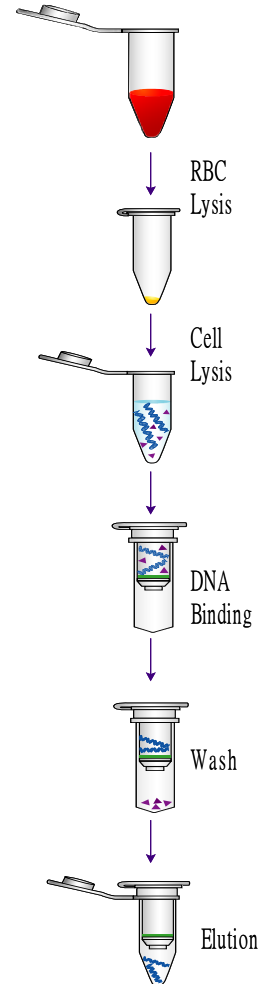
- (1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76

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

RBC Lysis Buffer is provided to remove non-nucleated red blood cells and reduce hemoglobin contamination. When the blood sample is less than 5 µl or the sample consists of nucleated blood cells, we recommend using the Cultured Cell Protocol to purify genomic DNA.

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: microcentrifuge tubes, absolute ethanol, RNase A (10 mg/ml)

Step 1 RBC Lysis	<p>Fresh blood</p> <ul style="list-style-type: none"> ● Collect fresh blood in EDTA-NA₂ treated collection tubes (or other anticoagulant mixtures). ● Transfer up to 300 µl of fresh blood to a 1.5 ml microcentrifuge tube. If the blood sample is more than 300 µl (up to 1 ml), add the sample to a sterile 15 ml centrifuge tube. ● Add 3 x the sample volume of RBC Lysis Buffer and mix by inversion. Do not vortex. ● Incubate the tube for 10 minutes at room temperature. ● Centrifuge for 5 minutes at 3,000 x g and remove the supernatant completely. ● Add 100 µl of RBC Lysis Buffer to resuspend the cell pellet.
Step 2 Cell Lysis	<ul style="list-style-type: none"> ● Add 200 µl of GB Buffer to the 1.5 ml microcentrifuge tube and mix by shaking vigorously. ● Incubate at 65°C to 70°C for 10 minutes or until 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) in a 70°C water bath (for Step 5 DNA Elution). <hr/> <p>Optional Step: RNA Degradation</p> <p>If RNA-free genomic DNA is required, perform this optional step.</p> <ul style="list-style-type: none"> ● Add 5 µl of RNase A (10 mg/ml) to the sample lysate and mix by vortex. ● Incubate at room temperature for 5 minutes.
Step 3 DNA Binding	<ul style="list-style-type: none"> ● Add 200 µl 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 Column in a 2 ml Collection Tube. ● Transfer all of 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 containing the flow-through and place the GD Column in a new 2 ml Collection Tube.
Step 4 Wash	<ul style="list-style-type: none"> ● Add 400 µl of W1 Buffer to the GD Column. ● Centrifuge at 14-16,000 x g for 30 seconds. ● Discard the flow-through and place the GD Column back in the 2 ml Collection Tube. ● Add 600 µl of Wash Buffer (ethanol added) to the GD Column. ● Centrifuge at 14-16,000 x g for 30 seconds. ● Discard the flow-through and 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 5 DNA Elution	<p>Standard elution volume is 100 µl. If less sample volume is 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.</p> <ul style="list-style-type: none"> ● Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. ● Add 100 µl of preheated Elution Buffer or TE to the center of the column matrix. ● Let stand for 3-5 minutes or until the Elution Buffer or TE is absorbed by the matrix. ● Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.



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

If blood samples have been frozen, it is recommended to use the following Frozen Blood Protocol.

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: Phosphate-Buffered Saline (PBS), Proteinase K (10 mg/ml), microcentrifuge tubes, absolute ethanol, RNase A (10 mg/ml)

<p>Step 1 Cell Lysis</p>	<ul style="list-style-type: none">● Add up to 200 μl of blood to a 1.5 ml microcentrifuge tube. If the sample volume is less than 200 μl add the appropriate volume of PBS.● Add 30 μl of Proteinase K (10 mg/ml) to the 1.5 ml microcentrifuge tube and mix briefly. Incubate the mixture at 60°C for 15 minutes.● Add 200 μl of GB Buffer to the 1.5 ml microcentrifuge tube and mix by shaking vigorously.● Incubate the mixture in a 70°C water bath for 15 minutes. During incubation, invert the tube every 3 minutes.● Preheat the required volume of Elution Buffer (200 μl/sample) in a 70°C water bath (for Step 4 DNA Elution). <hr/> <p>Optional Step: RNA Degradation If RNA-free genomic DNA is required, perform this optional step.</p> <ul style="list-style-type: none">● Add 5 μl of RNase A (10 mg/ml) to the sample lysate and mix by vortex.● Incubate the mixture at room temperature for 5 minutes.
<p>Step 2 DNA Binding</p>	<ul style="list-style-type: none">● Add 200 μl 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 Column in a 2 ml Collection Tube.● Transfer the entire mixture (including any precipitate) to the GD Column.● Centrifuge at 14-16,000 x g for 5 minutes.● Discard the 2 ml Collection Tube containing the flow-through and place the GD Column in a new 2 ml Collection Tube.
<p>Step 3 Wash</p>	<ul style="list-style-type: none">● Add 400 μl of W1 Buffer to the GD Column and centrifuge at 14-16,000 x g for 30 seconds.● Discard the flow-through and place the GD Column back in the 2 ml Collection Tube.● Add 600 μl of Wash Buffer (ethanol added) to the GD Column and centrifuge at 14-16,000 x g for 30 seconds.● Discard the flow-through and place the GD Column back in the 2 ml Collection Tube and centrifuge again at 14-16,000 x g for 3 minutes to dry the column matrix.
<p>Step 4 DNA Elution</p>	<p>Standard elution volume is 100 μl. If less sample volume is 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.</p> <ul style="list-style-type: none">● Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube.● Add 100 μl of preheated Elution Buffer or TE to the center of the column matrix.● Let stand for 3-5 minutes or until the Elution Buffer or TE is absorbed by the matrix● 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: RNase A (10 mg/ml), absolute ethanol, microcentrifuge tubes

Step 1 RBC Lysis	<ul style="list-style-type: none"> ● Transfer up to 200 μl of buffy coat to a 1.5 ml microcentrifuge tube. ● Add 3 x the sample volume of RBC Lysis Buffer to the tube and 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 and discard the supernatant completely. ● Add 500 μl of RBC Lysis Buffer to resuspend the white pellet. ● Centrifuge at 14-16,000 x g for 1 minute and discard the supernatant completely. ● Add 200 μl of RBC Lysis Buffer to the tube and resuspend the white pellet completely (Mix the tube by vortex only if the pellet is not resuspended completely and the column has become barred).
Step 2 Cell Lysis	<ul style="list-style-type: none"> ● Add 250 μl of GB Buffer to the 1.5 ml microcentrifuge tube and mix by shaking vigorously. ● Incubate at 65°C to 70°C for 30 minutes or until 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) in a 70°C water bath (for Step 5 DNA Elution). <p>Optional Step: RNA Degradation If RNA-free genomic DNA is required, perform this optional step.</p> <ul style="list-style-type: none"> ● Add 5 μl of RNase A (10 mg/ml) to the sample lysate and mix by vortex. ● Incubate at room temperature for 5 minutes.
Step 3 DNA Binding	<ul style="list-style-type: none"> ● Add 250 μl of absolute ethanol to the sample lysate and immediately mix by shaking vigorously 10 seconds. If precipitate appears, break it up by pipetting. ● Place a GD Column in a 2 ml Collection Tube. ● Transfer all of 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 containing the flow-through and place the GD Column in a new 2 ml Collection Tube.
Step 4 Wash	<ul style="list-style-type: none"> ● Add 400 μl of W1 Buffer to the GD Column. ● Centrifuge at 14-16,000 x g for 1 minute. ● Discard the flow-through and place the GD Column back in the 2 ml Collection Tube. ● Add 600 μl of Wash Buffer (ethanol added) to the GD Column. ● Centrifuge at 14-16,000 x g for 1 minute. ● Discard the flow-through and 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 5 DNA Elution	<p>Standard elution volume is 100 μl. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 μl.</p> <ul style="list-style-type: none"> ● Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. ● Add 100 μl of preheated Elution Buffer or TE to the center of the column matrix. ● Incubate the GD Column at 37°C in an incubator for 10 minutes. ● Centrifuge at 14-16,000 x g for 1 minute 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, absolute ethanol, RNase A (10 mg/ml)

<p>Step 1 Sample Preparation</p>	<p>Cultured animal cells</p> <p>If using adherent cells, trypsinize the cells before harvesting.</p> <ul style="list-style-type: none">● Transfer cells (up to 1×10^7) to a 1.5 ml microcentrifuge tube and harvest with centrifugation for 20 seconds at 6,000 x g.● Discard the supernatant and resuspend the cells with 150 µl of RBC Lysis Buffer. <p>Fresh blood (except human blood)</p> <p>For mammalian blood (non-nucleated), the sample volume can be up to 50 µl. For nucleated erythrocytes (eg. bird or fish), the sample volume can be up to 10 µl.</p> <ul style="list-style-type: none">● Add 150 µl of GT Buffer to a 1.5 ml microcentrifuge tube along with the blood sample and mix by shaking vigorously.
<p>Step 2 Lysis</p>	<ul style="list-style-type: none">● Add 200 µl of GB Buffer to the sample and mix by shaking vigorously for 5 seconds.● Incubate at 70°C for 10 minutes or until the sample lysate is clear. During incubation, invert the tube every 3 minutes. At this time, incubate the required Elution Buffer (200 µl per sample) at 70°C (for Step 5 DNA Elution). <hr/> <p>Optional Step: RNA Degradation</p> <p>If RNA free genomic DNA is required, perform this optional step.</p> <ul style="list-style-type: none">● After 70°C incubation, add 5 µl of RNase A (10 mg/ml) to the sample lysate and mix by vortex.● Incubate at room temperature for 5 minutes.
<p>Step 3 DNA Binding</p>	<ul style="list-style-type: none">● Add 200 µl of absolute ethanol to the sample lysate and immediately mix by shaking vigorously. If precipitate appears, break it up by pipetting.● Place a GD Column in a 2 ml Collection Tube.● Transfer all of 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 and place the GD Column in a new 2 ml Collection Tube.
<p>Step 4 Wash</p>	<ul style="list-style-type: none">● Add 400 µl of W1 Buffer to the GD Column.● Centrifuge at 14-16,000 x g for 30 seconds.● Discard the flow-through and place the GD Column back in the 2 ml Collection Tube.● Add 600 µl of Wash Buffer (ethanol added) to the GD Column.● Centrifuge at 14-16,000 x g for 30 seconds.● Discard the flow-through and 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.
<p>Step 5 DNA Elution</p>	<p>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.</p> <ul style="list-style-type: none">● Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube.● Add 100 µl of preheated Elution Buffer or TE to the center of the column matrix.● Let stand for 3-5 minutes or until the Elution Buffer or TE is absorbed by the matrix.● Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.

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

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: microcentrifuge tube, absolute ethanol, for gram-positive bacteria: lysozyme buffer (20 mg/ml lysozyme; 20 mM Tris-HCl; 2 mM EDTA; 1% Triton X-100; pH 8.0, prepare fresh lysozyme buffer immediately prior to use)

Step 1 Cell Harvesting/ Pre-lysis	<p>Gram-negative bacteria</p> <ul style="list-style-type: none"> ● Transfer cultured bacterial cells (up to 1×10^9) to a 1.5 ml microcentrifuge tube. ● Centrifuge for 1 minute at 14-16,000 x g and discard the supernatant. ● Add 200 µl of GT Buffer to the tube and resuspend the cell pellet by shaking vigorously or pipetting. ● Incubate at room temperature for 5 minutes. ● Proceed with the Lysis Step of the Cultured Cell Protocol. <p>Gram-positive bacteria</p> <ul style="list-style-type: none"> ● Transfer cultured bacterial cells (up to 1×10^9) to a 1.5 ml microcentrifuge tube. ● Centrifuge for 1 minute at 14-16,000 x g and discard the supernatant. ● Add 200 µl of lysozyme buffer to the tube and resuspend the cell pellet by shaking vigorously or pipetting. ● Incubate at room temperature for 10 minutes. During incubation, invert the tube every 2-3 minutes. ● Proceed with the Lysis Step of the Cultured Cell Protocol.
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Genomic DNA Mini Kit (Blood/Cultured Cell) Fungus Protocol

- Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to initial use
- Additional requirements: lyticase or zymolase, sorbitol buffer (1.2 M sorbitol; 10 mM CaCl₂; 0.1 M Tris-HCl pH 7.5; 35 mM mercaptoethanol)

Step 1 Cell Harvesting/ Pre-Lysis	<ul style="list-style-type: none"> ● Harvest fungus cells (up to 5×10^7) by centrifugation for 10 minutes at 5,000 x g. ● Discard the supernatant and resuspend the pellet in 600 µl of sorbitol buffer. ● Add 200 U of lyticase or zymolase. Incubate at 30°C for 30 minutes. ● Centrifuge the mixture for 10 minutes at 2,000 x g to harvest the spheroplast. ● Remove the supernatant and add 200 µl of GT Buffer to the tube and resuspend the cell pellet by shaking vigorously or pipetting. ● Incubate at room temperature for 5 minutes. ● Proceed with the Lysis Step of the Cultured Cell Protocol.
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Troubleshooting

Problem	Possible Reasons/Solution
Clogged Column	<p>Too much sample was used.</p> <ul style="list-style-type: none"> ● Reduce sample volume or separate into multiple tubes.
Low Yield	<p>Precipitate was formed at DNA Binding Step</p> <ul style="list-style-type: none"> ● Reduce the sample material. ● Prior to loading the column, break up precipitate in ethanol-added lysate. <p>Incorrect DNA Elution Step</p> <ul style="list-style-type: none"> ● Ensure that the Elution Buffer or TE is added to the center of the GD Column matrix and is absorbed completely.
Eluted DNA does not perform well in downstream applications	<p>Incomplete DNA Elution</p> <ul style="list-style-type: none"> ● Elute twice to increase yield. <p>Residual ethanol contamination</p> <ul style="list-style-type: none"> ● Following the Wash Step, dry the GD Column with additional centrifugation at 14-16,000 x g for 5 minutes or incubate at 60°C for 5 minutes. <p>Genomic DNA was degraded</p> <ul style="list-style-type: none"> ● Use fresh blood as long storage may result in fragmentation of genomic DNA.