

96-Well Genomic DNA Kit

For research use only

- Sample size** : up to 200 µl of blood, up to 20 mg of tissue, cultured animal cells (up to 1×10^7), bacterial cells (up to 1×10^9) and fungus cells (up to 5×10^7)
- Yield** : up to 30 µg/well
- Format** : 96-well plates
- Operation** : centrifuge
- Applications** : PCR, AFLP, RFLP, Southern Blotting, Real-time PCR

Geneaid



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Introduction

The 96-Well Genomic DNA Kit was designed for high-throughput purification of total DNA (including genomic, mitochondrial and viral DNA) from whole blood, animal tissue, cultured cells and fungus. Proteinase K and chaotropic salt are used to lyse cells and degrade protein, allowing DNA in the chaotropic salt to be easily bound by the glass fiber matrix (1) of each well of the plate. Once any contaminants have been removed, the purified 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. The kits can be used for manual filtration or with robotic handling systems and the purified DNA with approximately 20-30 Kb is suitable for use in PCR or other enzymatic reactions.

Quality Control

The quality of the 96-Well Genomic DNA Kit is tested on a lot-to-lot basis by isolating genomic DNA from 200 µl of fresh 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	GBP02	GBP04	GBP10
GT Buffer	60 ml	120 ml	240 ml
GB Buffer	60 ml	120 ml	240 ml
W1 Buffer	130 ml	130 ml	130 ml x 3
Wash Buffer* (Add Ethanol)	25 ml (100 ml)	50 ml (200 ml)	50 ml (200 ml x 3)
Elution Buffer	30 ml	60 ml	120 ml
Proteinase K** (Add ddH ₂ O)	45 mg (4.5 ml)	45 mg x 2 (4.5 ml x 2)	45 mg x 5 (4.5 ml x 5)
DNA Binding Plate	2 pcs	4 pcs	10 pcs
0.35 ml Collection Plate	2 pcs	4 pcs	10 pcs
Adhesive film	4 pcs	8 pcs	20 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 ddH₂O to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4°C (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.

96-Well Genomic DNA Kit Blood Protocol

- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
- Add ddH₂O to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4°C (see the bottle label for volume).
- Additional requirements: centrifugation system for 96-well plates, absolute ethanol, 2 ml collection plate, ddH₂O

Step 1 Cell Lysis	<ul style="list-style-type: none"> ➤ Add 200 µl of fresh blood and 20 µl of Proteinase K to each well of a 2 ml collection plate. ➤ Incubate at 60°C for 10 minutes. ➤ Add 200 µl of GB Buffer to each well and mix by shaking. ➤ Incubate at 60°C for 20 minutes or until the sample lysate is clear. ➤ Preheat the required Elution Buffer (100 µl per sample) to 60°C (for Step 4 DNA Elution).
Step 2 DNA Binding	<ul style="list-style-type: none"> ➤ Add 200 µl of absolute ethanol to each sample lysate in the 2 ml collection plate from Step 1. ➤ Mix immediately by pipetting 5-10 times. ➤ Place a DNA Binding Plate on a new 2 ml collection plate. ➤ Transfer the lysate mixture to the DNA Binding Plate. ➤ Centrifuge for 5 minutes at 1,000 x g.
Step 3 Wash	<ul style="list-style-type: none"> ➤ Add 300 µl of W1 Buffer to each well of the DNA Binding Plate. ➤ Centrifuge for 5 minutes at 1,000 x g. ➤ Add 600 µl of Wash Buffer (ethanol added) to each well of the DNA Binding Plate to wash again. ➤ Centrifuge for 5 minutes at 1,000 x g. ➤ Discard the flow-through and place the DNA Binding Plate back on the 2 ml collection plate. ➤ Centrifuge for 10 minutes at 1,000 x g to remove any ethanol residue.
Step 4 DNA Elution	<p>Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (50-100 µl) to increase DNA concentration.</p> <ul style="list-style-type: none"> ➤ Transfer the DNA Binding Plate to a clean 0.35 ml Collection Plate. ➤ Add 100 µl of preheated Elution Buffer or TE to the center of each well of the DNA Binding Plate. ➤ Let stand for 3 minutes or until the Elution Buffer or TE is absorbed by the matrix. ➤ Centrifuge for 5 minutes at 1,000 x g to elute the purified DNA.

96-Well Genomic DNA Kit Tissue Protocol

- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
- Add ddH₂O to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4°C (see the bottle label for volume).
- Additional requirements: centrifugation system for 96-well plates, 2 ml collection plate, RNase A, absolute ethanol

Step 1 Cell Lysis	<ul style="list-style-type: none"> ➤ Add 200 µl of GT Buffer and 20 µl of Proteinase K to each well of a 2 ml collection plate. ➤ Cut up to 20 mg of animal tissue (or 0.5 cm of mouse tail) and transfer it to each well of the 2 ml collection plate. ➤ Incubate the plate with shaking at 60°C for 1-2 hours to lyse the sample. ➤ Add 200 µl of GB Buffer to each well and mix by shaking. ➤ Incubate at 70°C for 20 minutes or until the sample lysate is clear. ➤ If RNA-free genomic DNA is required, add 5 µl of RNase A to each well and incubate at room temperature for 4 minutes. ➤ If there is insoluble material present following incubation, centrifuge the plate for 5 minutes at full speed and transfer the supernatant to a new 2 ml collection plate. ➤ Preheat the required Elution Buffer (50 µl per sample) to 70°C (for Step 4 DNA Elution).
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Step 2 DNA Binding	<ul style="list-style-type: none"> ➤ Add 200 µl of absolute ethanol to each sample lysate in the 2 ml collection plate from the previous step and mix immediately by pipetting 5-10 times. ➤ Place a DNA Binding Plate on a new 2 ml collection plate. ➤ Transfer the lysate mixture to the DNA Binding Plate. ➤ Centrifuge for 5 minutes at 1,000 x g.
Step 3 Wash	<ul style="list-style-type: none"> ➤ Add 300 µl of W1 Buffer to each well of the DNA Binding Plate. ➤ Centrifuge for 5 minutes at 1,000 x g. ➤ Add 600 µl of Wash Buffer (ethanol added) to each well of the DNA Binding Plate to wash again. ➤ Centrifuge for 5 minutes at 1,000 x g. ➤ Discard the flow-through and place the DNA Binding Plate back on the 2 ml collection plate. ➤ Centrifuge for 10 minutes at 1,000 x g to remove any ethanol residue.
Step 4 DNA Elution	<p>Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (50-100 µl) to increase DNA concentration.</p> <ul style="list-style-type: none"> ➤ Transfer the DNA Binding Plate to a clean 0.35 ml Collection Plate. ➤ Add 100 µl of preheated Elution Buffer or TE to the center of each well of the DNA Binding Plate. ➤ Let stand for 3 minutes or until the Elution Buffer or TE is absorbed by the matrix. ➤ Centrifuge for 5 minutes at 1,000 x g to elute the purified DNA.

96-Well Genomic DNA Kit Cultured Cell Protocol

- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
- Additional requirements: PBS (phosphate-buffered saline), 2 ml collection plates, absolute ethanol, RNase A (10 mg/ml)

Step 1 Sample Preparation	<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 each well of a 2 ml collection plate and harvest with centrifugation for 50 seconds at 5,000 x g. ➤ Discard the supernatant and resuspend the cells with 150 µl of PBS.
Step 2 Sample Lysis	<ul style="list-style-type: none"> ➤ Add 200 µl of GB Buffer to each well of the 2 ml collection plate and mix by shaking. ➤ Incubate the 2 ml collection plate at 60°C for 20 minutes or until the sample lysate is clear (during incubation shake the 2 ml collection plate every 2-3 minutes). ➤ If RNA-free genomic DNA is required, add 5 µl of RNase A to each well and incubate at room temperature for 4 minutes. ➤ Preheat the required Elution Buffer (100 µl per sample) at 60°C (for Step 5 DNA Elution).
Step 3 DNA Elution	<ul style="list-style-type: none"> ➤ Add 200 µl of absolute ethanol to each sample lysate in the 2 ml collection plate from the previous step and mix immediately by pipetting 5-10 times. ➤ Place a DNA Binding Plate on a new 2 ml collection plate. ➤ Transfer the lysate mixture to the DNA Binding Plate. ➤ Centrifuge for 5 minutes at 1,000 x g.
Step 4 Wash	<ul style="list-style-type: none"> ➤ Add 300 µl of W1 Buffer to each well of the DNA Binding Plate. ➤ Centrifuge for 5 minutes at 1,000 x g. ➤ Add 600 µl of Wash Buffer (ethanol added) into each well of the DNA Binding Plate to wash again. ➤ Centrifuge for 5 minutes at 1,000 x g. ➤ Discard the flow-through and place the DNA Binding Plate back on the 2 ml collection plate. ➤ Centrifuge for 10 minutes at 1,000 x g to remove the ethanol residue.
Step 5 DNA Elution	<p>Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (50-100 µl) to increase DNA concentration.</p> <ul style="list-style-type: none"> ➤ Transfer the DNA Binding Plate to a 0.35 ml Collection Plate. ➤ Add 100 µl of preheated Elution Buffer or TE into the center of each well of DNA Binding Plate. ➤ Let stand for 3 minutes or until the Elution Buffer or TE is absorbed by the matrix. ➤ Centrifuge for 5 minutes at 1,000 x g to elute the purified DNA.

96-Well Genomic DNA Kit Bacteria Protocol

- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
- Additional requirements: 2 ml collection plate, absolute ethanol, RNase A (10 mg/ml);
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)

<p>Step 1 Cell Harvesting/ Pre-lysis</p>	<p>Gram-negative bacteria</p> <ul style="list-style-type: none"> ● Transfer cultured bacterial cells (up to 1×10^9) to each well of a 2 ml collection plate. ● Centrifuge for 3 minutes at full speed and discard the supernatant. ● Add 200 μl of GT Buffer to each well and resuspend the cell pellet by 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 each well of a 2 ml collection plate. ● Centrifuge for 3 minutes at full speed and discard the supernatant. ● Add 200 μl of lysozyme buffer to each well and resuspend the cell pellet by pipetting. ● Incubate at room temperature for 10 minutes. During incubation, shake the 2 ml collection plate every 2-3 minutes. ● Proceed with the Lysis Step of the Cultured Cell Protocol.
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96-Well Genomic DNA Kit Fungus Protocol

- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
- Additional requirements: 2 ml collection plate, RNase A (10 mg/ml), lyticase or zymolase, absolute ethanol, sorbitol buffer (1.2 M sorbitol; 10 mM CaCl₂; 0.1 M Tris-HCl pH 7.5; 35 mM mercaptoethanol)

<p>Step 1 Cell Harvesting/ Prelysis</p>	<ul style="list-style-type: none"> ● Harvest fungus cells (up to 5×10^7) in each well of a 2 ml collection plate 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 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 each well of a 2 ml collection plate and resuspend the cell pellet by 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 the sample volume.
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 plate, break up the precipitate in the 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 each well of the DNA Binding Plate and is absorbed completely. ● Ensure to preheat the Elution Buffer or TE prior to adding to the DNA Binding Plate.
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 plate with additional vacuum or incubate at 60°C for 5 minutes.