HiYield™ 96-Well Genomic DNA Extraction Kit

Cat. No.:	YGB96B-2	YGB96B-4	YGB96B-10
Product Name:	HiYield™ 96-Well Genomic DNA Extraction Kit		
Reactions:	2	4	10
Format:	96-Well Plates		
Binding Capacity:	Up to 30 μg/well		
Operation:	Centrifuge		
Operation Time:	Within 60 Minutes		

Introduction

HiYield™ 96-Well Genomic DNA Extraction Kit is designed for high-throughput purification of total DNA (including genomic, mitochondrial and viral DNA) from whole blood, plasmid, serum, buffy coat, bacteria and a variety of animal tissues or cells. The entire procedure can be completely in one hour without phenol/chloroform extraction and alcohol precipitation. This kit can be used for manual filtration or with robotic handing systems. The purified DNA (approximately 20-30kb) is suitable for PCR or other enzymatic reactions.

Features

High-throughput purification of genomic DNA within 1 hour.
Reproducible recoveries for constant results.
Complete removal of all contaminants for reliable downstream applications.

Applications

Purified DNA is ready for direct use in PCR, Southern Blotting, Real-Time PCR, AFLP, RFLP, PADP.

Quality Control

The quality of HiYield™ 96-Well Genomic DNA Kit is tested on a lot-to-lot basis by isolation of genomic DNA from 200 μl of fresh human whole blood. Purified DNA is quantified with a spectrophotometer and the yield of genomic DNA is more than 5 ug with A260/A280 ratio 1.7 - 1.9. The purified DNA is checked by electrophoresis.

Contents

ITEM	YGB96B-2	YGB96B-4	YGB96B-10
Genomic DNA Binding Plate	2 plates	4 plates	10 plates
Adhesive Film	4 pcs	8 pcs	20 pcs
350 µl Collection Plate	2 plates	4 plates	10 plates
GT Buffer	60 ml	120 ml	240 ml
GB Buffer	60 ml	120 ml	240 ml
W1 Buffer	130 ml	130 ml	390 ml
Wash Buffer(concentrated)	25 ml	50 ml	150 ml
Elution Buffer	30 ml	60 ml	120 ml
Proteinase K	45 mg	90 mg	225 mg

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

**Add ddH_20 to prepare the 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.

Blood Protocol

Additional Requirements:
Centrifugation system for 96-well plates, absolute ethanol, 2 ml collection plate, ddH_20.

Things Before Starting:
Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
Add ddH_20 to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4ºC (see the bottle label for volume).

Step1 Cell Lysis	★Add 200µl of fresh blood and **20 µl of Proteinase K** to each well of a 2 ml collection plate. ★Incubate at 60ºC for 10 minutes. ★Add **200 µl of GB Buffer** to each well and mix by shaking. ★Incubate at 60ºC for 20 minutes or until the sample lysate is clear. ★Preheat the required **Elution Buffer** (100µl per sample) to 60 ºC (for Step 4 DNA Elution).
Step 2 DNA Binding	★Add 200 µl of absolute ethanol to each sample lysate in the 2 ml collection plate from the previous step. ★Mix immediately by pipetting 5-10 times. ★Place a **Genomic DNA Binding Plate** on a new 2 ml collection plate. ★Transfer the lysate mixture to the **Genomic DNA Binding Plate**. ★Centrifuge for 5 minutes at 1,000 x g.
Step 3 Wash	★Add **300 µl of W1 Buffer** to each well of the **Genomic 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 **Genomic DNA Binding Plate** to wash again. ★Centrifuge for 5 minutes at 1,000 x g. ★Discard the flow-through and place the **Genomic 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	★Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (50-100 µl) to increase DNA concentration. ★Transfer the **Genomic DNA Binding Plate** to a clean **350 ul Collection Plate**. ★Add **100 µl of preheated Elution Buffer** or TE to the center of each well of the **Genomic 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.

Tissue Protocol

Additional Requirements:

Centrifugation system for 96-well plates, 2ml collection plate, RNase A, absolute ethanol.

Things Before Starting:

Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume). Add dd$H_2$0 to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4ºC (see the bottle label for volume).

Step1 Cell Lysis	★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 60ºC for 1-2 hours to lyse the sample. ★Add **200 µl of GB Buffer** to each well and mix by shaking. ★Incubate at 70ºC 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 the 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 70ºC (for Step 4 DNA Elution).

Step 2 DNA Binding	★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 **Genomic DNA Binding Plate** on a new 2 ml collection plate. ★Transfer the lysate mixture to the **Genomic DNA Binding Plate**. ★Centrifuge for 5 minutes at 1,000 x g.
Step 3 Wash	★Add **300 µl of W1 Buffer** to each well of the **Genomic 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 **Genomic DNA Binding Plate** to wash again. ★Centrifuge for 5 minutes at 1,000 x g. ★Discard the flow-through and place the **Genomic 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	★Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (50-100 µl) to increase DNA concentration. ★Transfer the **Genomic DNA Binding Plate** to a clean **350 ul Collection Plate**. ★Add **100 µl of preheated Elution Buffer** or TE to the center of each well of the **Genomic 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.

Cultured Cell Protocol

Additional Requirements:

PBS (phosphate-buffered saline), 2 ml collection plates, absolute ethanol, RNase A (10 mg/ml)

Things Before Starting:

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

Step1 Sample Preparation	★If using adherent cells, trypsinize the cells before harvesting. ★Transfer cells (up to 1 x 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	★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 60ºC 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 60ºC (for Step 5 DNA Elution).
Step 3 DNA Elution	★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 **Genomic DNA Binding Plate** on a new 2 ml collection plate. ★Transfer the lysate mixture to the **Genomic DNA Binding Plate**. ★Centrifuge for 5 minutes at 1,000 x g.
Step 4 Wash	★Add **300 µl of W1 Buffer** to each well of the **Genomic 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 **Genomic DNA Binding Plate** to wash again. ★Centrifuge for 5 minutes at 1,000 x g. ★Discard the flow-through and place the **Genomic 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	★Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (50-100 µl) to increase DNA concentration. ★Transfer the **Genomic DNA Binding Plate** to a **350 ul Collection Plate**. ★Add **100µl of preheated Elution Buffer** or TE into the center of each well of **Genomic 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.

Bacteria Protocol

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**.

Things Before Starting:

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

Step1 Cell Harvesting / Pre-lysis	**Gram-negative bacteria** ★Transfer cultured bacterial cells (up to 1 x 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. **Gram-positive bacteria** ★Transfer cultured bacterial cells (up to 1 x 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.
Step 2 Sample Lysis	★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 60ºC 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 60ºC (for Step 5 DNA Elution).

Step 3 DNA Elution	★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 **Genomic DNA Binding Plate** on a new 2 ml collection plate. ★Transfer the lysate mixture to the **Genomic DNA Binding Plate**. ★Centrifuge for 5 minutes at 1,000 x g.
Step 4 Wash	★Add **300 µl of W1 Buffer** to each well of the **Genomic 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 **Genomic DNA Binding Plate** to wash again. ★Centrifuge for 5 minutes at 1,000 x g. ★Discard the flow-through and place the **Genomic 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	★Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (50-100 µl) to increase DNA concentration. ★Transfer the **Genomic DNA Binding Plate** to a **350 ul Collection Plate**. ★Add **100µl of preheated Elution Buffer** or TE into the center of each well of **Genomic 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.

Fungus Protocol

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)

Things Before Starting:

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

Step1 Cell Harvesting / Pre-lysis	★Harvest fungus cells (up to 5 x 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 30ºC 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.
Step 2 Sample Lysis	★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 60ºC 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 60ºC (for Step 5 DNA Elution).
Step 3 DNA Elution	★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 **Genomic DNA Binding Plate** on a new 2 ml collection plate. ★Transfer the lysate mixture to the **Genomic DNA Binding Plate**. ★Centrifuge for 5 minutes at 1,000 x g.
Step 4 Wash	★Add **300 µl of W1 Buffer** to each well of the **Genomic 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 **Genomic DNA Binding Plate** to wash again. ★Centrifuge for 5 minutes at 1,000 x g. ★Discard the flow-through and place the **Genomic 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	★Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (50-100 µl) to increase DNA concentration. ★Transfer the **Genomic DNA Binding Plate** to a **350 ul Collection Plate**. ★Add **100µl of preheated Elution Buffer** or TE into the center of each well of **Genomic 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.

Troubleshooting

Problem	Possible Reasons/Solution
Clogged Column	**Too much sample was used** →Reduce the sample volume.
Low Yield	**Precipitate was formed at DNA Binding Step** →Reduce the sample material. →Prior to loading the plate, break up the precipitate in the ethanol-added lysate. **Incorrect DNA Elution Step** →Ensure that the Elution Buffer or TE is added to the center of each well of the Genomic DNA Binding Plate and is absorbed completely. →Ensure to preheat the Elution Buffer or TE prior to adding to the Genomic DNA Binding Plate.
Eluted DNA does not perform well in downstream applications.	**Incomplete DNA Elution** →Elute twice to increase yield. **Residual ethanol contamination** →Following the Wash Step, dry the plate with additional vacuum or incubate at 60ºC for 5 minutes.