HiYield Genomic DNA Midi Kit (Fresh Blood / Cultured Cells)

Cat. No.:	YGBI25
Product Name:	HiYield Genomic DNA Midi Kit (Fresh Blood / Cultured Cells)
Reactions:	25
Sample:	Up to 3 ml of fresh whole blood, 5 x 10^7 of cultured animal cells
Yield:	Up to 100 ug
Format:	Spin Columns
Operation:	Centrifuge
Operation Time:	60 Minutes

Introduction

HiYield Genomic DNA Midi Kit (Fresh Blood/Cultured Cells) provides a fast and economical method for purification of total DNA (including genomic, mitochondrial and viral DNA) from fresh whole blood and cultured animal cells. The entire procedure can be completed in 60 minutes without phenol/chloroform extraction or alcohol precipitation, with an average DNA yield of 60 µg from 3 ml of fresh whole blood. Purified DNA, with approximately 20-30 Kb, is suitable for direct use in PCR or other enzymatic reactions.

Features

Complete removal of all contaminants for reliable downstream applications.
No phenol, chloroform or alcohol.
Rapid and simple procedure.

Contents

ITEM	YGBI25
RBC Lysis Buffer	360 ml
GB Buffer	60 ml
W1 Buffer	60 ml
Wash Buffer*	25 ml
Elution Buffer	30 ml
GBI Column	25 pcs

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

Applications

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

Quality Control

The quality of HiYield Genomic DNA Midi Kit (Fresh Blood/Cultured Cells) is tested on a lot-to-lot basis by isolation of genomic DNA from 3 ml of fresh human whole blood. Purified DNA is quantified with a spectrophotometer and the yield of genomic DNA is more than 50 ug with A260/A280 ratio 1.7 - 1.9. The purified DNA is checked by electrophoresis.

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.

Fresh Blood Protocol

Additional Requirements:

Centrifuge tubes, absolute ethanol, RNase A (10 mg/ml).

Things Before Starting:

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

Step1 **RBC Lysis**	★Collect fresh blood in EDTA-NA2 treated collection tubes (or other anticoagulant mixtures). ★Transfer up to 3 ml of fresh blood to a 50 ml centrifuge tube. ★Add 3 volumes of **RBC Lysis Buffer** to 1 volume of the sample and mix by inversion. (For example, add 9 ml of RBC Lysis Buffer to 3 ml of fresh blood.) 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 **1 ml of RBC Lysis Buffer** to resuspend the cell pellet.
Step2 **Cell Lysis**	★Add **2 ml of GB Buffer** to the tube and mix by vortex. ★Incubate the mixture at room temperature for 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes. ★At this time, preheat the required **Elution Buffer** (1 ml per sample) in a 70ºC water bath (for Step 5 DNA Elution).
	Optional Step: RNA Degradation If RNA-free genomic DNA is required, perform this optional step. ★Add 20 µl of RNase A (10 mg/ml) to the sample lysate and mix by vortex. ★Incubate at room temperature for 10 minutes.

Step 3 **DNA Binding**	★Add 2 ml of absolute ethanol to the sample lysate and vortex immediately for 10 seconds. If precipitate appears, break it up by pipetting. ★Place a **GBI Column** in a 50 ml centrifuge tube. ★Transfer all of the mixture (including any precipitate) from previous step to the **GBI Column**. ★Close the cap and centrifuge at 4,000 x g for 5 minutes. ★Discard the flow-through and place the **GBI Column** in a new 50 ml centrifuge tube
Step 4 **Wash**	★Add **4 ml of W1 Buffer** into the **GBI Column**. ★Centrifuge at 4,000 x g for 3 minutes. ★Discard the flow-through and place the **GBI Column** back in the 50 ml centrifuge tube. ★Add **6 ml of Wash Buffer** (ethanol added) to the **GBI Column**. ★Centrifuge at 4,000 x g for 3 minutes to wash again. ★Discard the flow-through and place the **GBI Column** back in the 50 ml centrifuge tube. ★Centrifuge at 4,000 x g for 10 minutes to dry the column matrix.
Step 5 **DNA Elution**	★Standard elution volume is 500 µl. If higher DNA yield is required, repeat the DNA Elution Step to increase DNA recovery and the total elution volume to about 1 ml. ★Transfer the dried **GBI Column** to a clean 50 ml centrifuge tube. ★Add **500 µl of preheated Elution Buffer** or TE into the center of the column matrix. ★Incubate at 60ºC for 3 minutes. ★Centrifuge at 4,000 x g for 2 minutes at room temperature to elute the purified DNA.

Cultured Cells Protocol

Additional Requirements:

Centrifuge tubes, absolute ethanol, RNase A (10 mg/ml).

Things Before Starting:

Add absolute ethanol to 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 5 x 10^7) to a 50 ml centrifuge tube and harvest with centrifugation for 5 minutes at 6,000 x g. ★Discard the supernatant and resuspend the cells in 1.5 ml of RBC Lysis Buffer.
Step2 Cell Lysis	★Add **2 ml of GB Buffer** to the tube and mix by vortex. ★Incubate the mixture in a 70ºC water bath for 20 minutes. During incubation, invert the tube every 3 minutes. At this time, preheat the required **Elution Buffer** (1 ml per sample) in a 70ºC water bath (for Step 5 DNA Elution).
	Optional Step: RNA Degradation If RNA-free genomic DNA is required, perform this optional step. ★Add 20 µl of RNase A (10 mg/ml) to the sample lysate and mix by vortex. ★Incubate at room temperature for 10 minutes.
Step 3 DNA Binding	★Add 2 ml of absolute ethanol to the sample lysate and vortex immediately for 10 seconds. If precipitate appears, break it up by pipetting. ★Place a **GBI Column** in a 50 ml centrifuge tube. ★Transfer all of the mixture (including any precipitate) to the **GBI Column**. ★Close the cap and centrifuge at 4,000 x g for 5 minutes. ★Discard the flow-through and place the **GBI Column** in a new 50 ml centrifuge tube.

Step 4 **Wash**	★Add **4 ml of W1 Buffer** into the **GBI Column**. ★Centrifuge at 4,000 x g for 3 minutes. ★Discard the flow-through and place the **GBI Column** back in the 50 ml centrifuge tube. ★Add **6 ml of Wash Buffer** (ethanol added) to the **GBI Column**. ★Centrifuge at 4,000 x g for 3 minutes to wash again. ★Discard the flow-through and place the **GBI Column** back in the 50 ml centrifuge tube. ★Centrifuge at 4,000 x g for 10 minutes to dry the column matrix.
Step 5 **DNA Elution**	★Standard elution volume is 500 µl. If higher DNA yield is required, repeat the DNA Elution Step to increase DNA recovery and the total elution volume to about 1 ml. ★Transfer the dried **GBI Column** to a clean 50 ml centrifuge tube. ★Add **500 µl of preheated Elution Buffer** or TE into the center of the column matrix. ★Incubate at 60ºC for 3 minutes. ★Centrifuge at 4,000 x g for 2 minutes at room temperature to elute the purified DNA.

Troubleshooting

Problem	Possible reason/ Solution
Column clogged	**Too much sample was used.** ★Reduce sample volume or separate into multiple tubes.
	Precipitate was formed at DNA Binding Step. ★Reduce the sample material. ★Prior to loading the column, break up precipitate in ethanol-added lysate.
Low yield	**Incorrect DNA Elution Step.** ★Ensure that Elution Buffer was added and absorbed to the center of GBI Column matrix.
	Incomplete DNA Elution. ★Elute twice to increase yield.
Eluted DNA does not perform well in downstream applications.	**Residual ethanol contamination.** ★Following the wash step, dry GBI Column with additional centrifugation at full speed for 10 minutes or incubation at 60℃ for 10 minutes.
	RNA contamination. ★Perform Optional RNA degradation Step.
	Genomic DNA was degraded. ★Use fresh blood, long storage may result in fragmentation of genomic DNA.