

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

Cat. No.:	YGBM10	YGBM25
Product Name:	HiYield Genomic DNA Maxi Kit (Fresh Blood / Cultured Cells)	
Reactions:	10	25
Sample:	Up to 10 ml of fresh whole blood, 1×10^8 of cultured animal cells	
Yield:	Up to 200 ug	
Format:	Spin Columns	
Operation:	Centrifuge	
Operation Time:	60 Minutes	

Introduction

HiYield Genomic DNA Maxi 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 150 µg from 10 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	YGBM10	YGBM25
RBC Lysis Buffer	360 ml	1000 ml
GB Buffer	60 ml	130 ml
W1 Buffer	45 ml	130 ml
Wash Buffer*	25 ml	50 ml
Elution Buffer	30 ml	60 ml
GBI Column	10 pcs	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 Maxi Kit (Fresh Blood/Cultured Cells) is tested on a lot-to-lot basis by isolation of genomic DNA from 10 ml of fresh human whole blood. Purified DNA is quantified with a spectrophotometer and the yield of genomic DNA is more than 80 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).

<p style="text-align: center;">Step1 RBC Lysis</p>	<ul style="list-style-type: none"> ★Collect fresh blood in EDTA-NA2 treated collection tubes (or other anticoagulant mixtures). ★Transfer up to 10 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 30 ml of RBC Lysis Buffer to 10 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 4 ml of RBC Lysis Buffer to resuspend the cell pellet.
<p style="text-align: center;">Step2 Cell Lysis</p>	<ul style="list-style-type: none"> ★Add 10 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 (2 ml per sample) in a 70°C water bath (for Step 5 DNA Elution). <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 50 µl of RNase A (10 mg/ml) to the sample lysate and mix by vortex. ★Incubate at room temperature for 10 minutes.

<p>Step 3 DNA Binding</p>	<ul style="list-style-type: none"> ★Add 10 ml of absolute ethanol to the sample lysate and vortex immediately for 10 seconds. If precipitate appears, break it up by pipetting. ★Place a GBM Column in a 50 ml centrifuge tube. ★Transfer all of the mixture (including any precipitate) from previous step to the GBM Column. ★Close the cap and centrifuge at 4,000 x g for 5 minutes. ★Discard the flow-through and place the GBM Column in a new 50 ml centrifuge tube
<p>Step 4 Wash</p>	<ul style="list-style-type: none"> ★Add 4 ml of W1 Buffer into the GBM Column. ★Centrifuge at 4,000 x g for 3 minutes. ★Discard the flow-through and place the GBM Column back in the 50 ml centrifuge tube. ★Add 6 ml of Wash Buffer (ethanol added) to the GBM Column. ★Centrifuge at 4,000 x g for 3 minutes to wash again. ★Discard the flow-through and place the GBM Column back in the 50 ml centrifuge tube. ★Centrifuge at 4,000 x g for 10 minutes to dry the column matrix.
<p>Step 5 DNA Elution</p>	<ul style="list-style-type: none"> ★Standard elution volume is 1 ml. If higher DNA yield is required, repeat the DNA Elution Step to increase DNA recovery and the total elution volume to about 2 ml. ★Transfer the dried GBM Column to a clean 50 ml centrifuge tube. ★Add 1 ml of preheated Elution Buffer or TE into the center of the column matrix. ★Incubate at 60°C for 3 minutes. ★Centrifuge at 4,000 x g for 5 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).

<p style="text-align: center;">Step1 Sample Preparation</p>	<p>If using adherent cells, trypsinize the cells before harvesting.</p> <ul style="list-style-type: none"> ★ Transfer cells (up to 1×10^8) 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 5 ml of RBC Lysis Buffer.
<p style="text-align: center;">Step2 Cell Lysis</p>	<ul style="list-style-type: none"> ★ Add 10 ml of GB Buffer to the tube and mix by vortex. ★ Incubate the mixture in a 70°C water bath for 20 minutes. During incubation, invert the tube every 3 minutes. At this time, preheat the required Elution Buffer (2 ml per sample) in a 70°C water bath (for Step 5 DNA Elution). <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 50 µl of RNase A (10 mg/ml) to the sample lysate and mix by vortex. ★ Incubate at room temperature for 10 minutes.
<p style="text-align: center;">Step 3 DNA Binding</p>	<ul style="list-style-type: none"> ★ Add 10 ml of absolute ethanol to the sample lysate and vortex immediately for 10 seconds. If precipitate appears, break it up by pipetting. ★ Place a GBM Column in a 50 ml centrifuge tube. ★ Transfer all of the mixture (including any precipitate) to the GBM Column. ★ Close the cap and centrifuge at 4,000 x g for 5 minutes. ★ Discard the flow-through and place the GBM Column in a new 50 ml centrifuge tube.

<p>Step 4 Wash</p>	<ul style="list-style-type: none"> ★Add 4 ml of W1 Buffer into the GBM Column. ★Centrifuge at 4,000 x g for 3 minutes. ★Discard the flow-through and place the GBM Column back in the 50 ml centrifuge tube. ★Add 6 ml of Wash Buffer (ethanol added) to the GBM Column. ★Centrifuge at 4,000 x g for 3 minutes to wash again. ★Discard the flow-through and place the GBM Column back in the 50 ml centrifuge tube. ★Centrifuge at 4,000 x g for 10 minutes to dry the column matrix.
<p>Step 5 DNA Elution</p>	<ul style="list-style-type: none"> ★Standard elution volume is 1 ml. If higher DNA yield is required, repeat the DNA Elution Step to increase DNA recovery and the total elution volume to about 2 ml. ★Transfer the dried GBM Column to a clean 50 ml centrifuge tube. ★Add 1 ml of preheated Elution Buffer or TE into the center of the column matrix. ★Incubate at 60°C for 3 minutes. ★Centrifuge at 4,000 x g for 5 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 GBM 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 GBM Column with additional centrifugation at full speed for 10 minutes or incubation at 60°C 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.