# HiYield Genomic DNA Maxi Kit (Frozen Blood / Cultured Cells)

| Cat. No.: | YGDM10 | YGDM25 |
|---|---|---|
| **Product Name:** | HiYield Genomic DNA Maxi Kit (Frozen Blood / Cultured Cells) | |
| **Reactions:** | 10 | 25 |
| **Sample:** | Up to 10 ml of frozen whole blood, 1 x $10^8$ of cultured animal cells | |
| **Yield:** | Up to 150 ug | |
| **Format:** | Spin Columns | |
| **Operation:** | Centrifuge | |
| **Operation Time:** | 60 Minutes | |

## Introduction

HiYield Genomic DNA Maxi Kit (Frozen Blood/Cultured Cells) provides a fast and economical method for purification of total DNA (including genomic, mitochondrial and viral DNA) from frozen 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 140 µg from 10 ml of frozen 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 | YGDM10 | YGDM25 |
|---|---|---|
| GB Buffer | 120 ml | 280 ml |
| W1 Buffer | 45 ml | 130 ml |
| Wash Buffer* | 25 ml | 50 ml |
| Proteinase K** | 55 mg | 135 mg |
| Elution Buffer | 30 ml | 60 ml |
| GDM Column | 10 pcs | 25 pcs |

*Add absolute ethanol to Wash Buffer prior to initial use (see the bottle label for volume).
**Add $ddH_20$ to prepare Proteinase K (vortex to dissolve and spin down) and store at 4ºC (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 (Frozen Blood/Cultured Cells) is tested on a lot-to-lot basis by isolation of genomic DNA from 10 ml of frozen 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.

# Frozen 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).

Add dd$H_2$0 to prepare Proteinase K (vortex to dissolve and spin down) and store at 4ºC (see the bottle label for volume).

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| **Step1 Cell Lysis** | ★ Add **500 µl of Proteinase K** and up to 10 ml of blood to a 50 ml centrifuge tube and mix briefly.<br>★ Incubate the mixture at 60ºC for 15 minutes. During incubation, invert the tube every 3 minutes.<br>★Add **10 ml of GB Buffer** to the tube and mix by vortex.<br>★Incubate the mixture in a 70ºC water bath for 20 minutes. During incubation, invert the tube every 3-5 minutes.<br>★At this time, preheat the required **Elution Buffer** (2 ml per sample) in a 70ºC water bath (for Step 4 DNA Elution). |
| | **Optional Step: RNA Degradation**<br>If RNA-free genomic DNA is required, perform this optional step.<br>★Add 50 µl of RNase A (10 mg/ml) to the sample lysate and mix by vortex.<br>★Incubate at room temperature for 10 minutes. |
| **Step 2 DNA Binding** | ★Add 10 ml of absolute ethanol to the sample lysate and vortex immediately for 10 seconds.<br>★Place a **GDM Column** in a 50 ml centrifuge tube.<br>★Transfer all of the mixture (including any precipitate) to the **GDM Column**.<br>★Close the cap and centrifuge at 4,000 x g for 5 minutes. |
| **Step 3 Wash** | ★ Add **4 ml of W1 Buffer** into the **GDM Column**.<br>★Centrifuge at 4,000 x g for 3 minutes.<br>★Discard the flow-through and place the **GDM Column** back in the 50 ml centrifuge tube.<br>★Add **6 ml of Wash Buffer** (ethanol added) to the **GDM Column**.<br>★Centrifuge at 4,000 x g for 3 minutes to wash again. |

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| | ★Discard the flow-through and place the **GDM Column** back in the 50 ml centrifuge tube.<br>★Centrifuge at 4,000 x g for 10 minutes to dry the column matrix. |
| **Step 4**<br>**DNA Elution** | ★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.<br>★Transfer the dried **GDM Column** to a clean 50 ml centrifuge tube.<br>★Add **1 ml of preheated Elution Buffer** or TE into the center of the column matrix.<br>★Incubate at 60ºC for 3 minutes.<br>★Centrifuge at 4,000 x g for 5 minutes at room temperature to elute the purified DNA. |

## Cultured Cells Protocol

**Additional Requirements:**

PBS (phosphate-buffered saline), 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).

Add dd$H_2$0 to prepare Proteinase K (vortex to dissolve and spin down) and store at 4ºC (see the bottle label for volume).

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| **Step1**<br>**Cell Lysis** | **If using adherent cells, trypsinize the cells before harvesting.**<br>★Transfer cells (up to 1 x $10^8$) to a 50 ml centrifuge tube and harvest with centrifugation for 5 minutes at 4,000 x g.<br>★Resuspend the cells with 10 ml of PBS.<br>★Add **500 µl of Proteinase K** to a 50 ml centrifuge tube and mix briefly.<br>★Add **10 ml of GB Buffer** to the 50 ml centrifuge tube and mix by vortex.<br>★Incubate the mixture in a 70ºC water bath for 20 minutes. During incubation, invert the tube every 3-5 minutes.<br>★At this time, preheat the required **Elution Buffer** (2 ml per sample) in a 70ºC water bath (for Step 4 DNA Elution). |

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| | **Optional Step: RNA Degradation**<br>If RNA-free genomic DNA is required, perform this optional step.<br>★Add 50 µl of RNase A (10 mg/ml) to the sample lysate and mix by vortex.<br>★Incubate at room temperature for 10 minutes. |
| **Step 2**<br>**DNA Binding** | ★Add 10 ml of absolute ethanol to the sample lysate and vortex immediately for 10 seconds. If precipitate appears, break it up by pipetting.<br>★Place a **GDM Column** in a 50 ml centrifuge tube.<br>★Transfer 15 ml of the mixture (including any precipitate) to the **GDM Column**.<br>★Close the cap and centrifuge at 4,000 x g for 5 minutes.<br>★Discard the flow-through and place the **GDM Column** back in the 50 ml centrifuge tube.<br>★Transfer the remaining mixture to the **GDM Column**.<br>★Centrifuge at 4,000 x g for 5 minutes.<br>★Discard the flow-through and place the **GDM Column** back in the 50 ml centrifuge tube. |
| **Step 3**<br>**Wash** | ★ Add **4 ml of W1 Buffer** into the **GDM Column**.<br>★Centrifuge at 4,000 x g for 3 minutes.<br>★Discard the flow-through and place the **GDM Column** back in the 50 ml centrifuge tube.<br>★Add **6 ml of Wash Buffer** (ethanol added) to the **GDM Column**.<br>★Centrifuge at 4,000 x g for 3 minutes to wash again.<br>★Discard the flow-through and place the **GDM Column** back in the 50 ml centrifuge tube.<br>★Centrifuge at 4,000 x g for 10 minutes to dry the column matrix. |
| **Step 4**<br>**DNA Elution** | ★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.<br>★Transfer the dried **GDM Column** to a clean 50 ml centrifuge tube.<br>★Add **1 ml of preheated Elution Buffer** or TE into the center of the column matrix.<br>★Incubate at 60ºC for 3 minutes.<br>★Centrifuge at 4,000 x g for 5 minutes at room temperature to elute the purified DNA. |

**Troubleshooting**

| Problem | Possible reason/ Solution |
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| **Column clogged** | **Too much sample was used.**<br>★Reduce sample volume or separate into multiple tubes. |
| | **Precipitate was formed at DNA Binding Step.**<br>★Reduce the sample material.<br>★Prior to loading the column, break up precipitate in ethanol-added lysate. |
| **Low yield** | **Incorrect DNA Elution Step.**<br>★ Ensure that Elution Buffer or TE is added to the center of GDM Column matrix and is absorbed completely.<br>★ Ensure to preheat the Elution Buffer or TE prior to adding to the GDM Column. |
| | **Incomplete DNA Elution.**<br>★Elute twice to increase yield. |
| **Eluted DNA does not perform well in downstream applications.** | **Residual ethanol contamination.**<br>★Following the wash step, dry GDM Column with additional centrifugation at full speed for 10 minutes or incubation at 60℃ for 10 minutes. |
| | **RNA contamination.**<br>★Perform Optional RNA degradation Step. |