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HiYield™ Genomic DNA Isolation Kit (Blood)

Cat. No.:	YGD100	YGD1000
Product Name:	HiYield™ Genomic DNA Isolation Kit (Blood)	
Reactions:	100	1000
Sample:	300 µl of whole blood, 3 ml of whole blood, 10 ml of whole blood, 10 ml of compromised whole blood	
Yield:	High yield and high quality DNA with A260/A280 = 1.8-2.0	
Elution Volume:	100 µl-1 ml (varies according to starting sample amount between 300 µl-10 ml)	
Format:	Reagent (scalable for a wide range of sample sizes)	
Operation:	Centrifuge	
Operation Time:	Within 40 minutes for 300 µl of whole blood (including DNA rehydration)	

Description

HiYield™ Genomic DNA Isolation Kit (Blood) is designed specifically for isolating high molecular weight genomic, mitochondrial or viral DNA from large volumes of whole blood in a scalable, simple-to-use format. Whole blood samples are first treated with RBC Lysis Buffer to remove non-nucleated red blood cells and to reduce hemoglobin contamination. Cells are lysed with Cell Lysis Buffer (a DNA stabilizer) which limits the activity of intracellular DNases and also DNases found elsewhere in the environment. RNA is then removed by treatment with an RNA digesting enzyme and protein is then removed from the lysate with the Protein Removal Buffer. Finally, the genomic DNA is recovered by precipitation with alcohol and dissolved in hydration solution. The high-quality purified DNA can be utilized for a wide range of downstream applications. This scalable purification kit fulfills the need for high quality nucleic acid, reproducible purification, ease of use and increased throughput for research laboratories.

Features

Scalable purification procedure is convenient and flexible for a wide range of sample sizes.
Isolation of high-quality DNA from whole blood within 40 minutes.
Complete removal of all contaminants for reliable downstream applications.
A complete solution for sample-to-storage purification.

Applications

Purified DNA is highly stable and ready for use in a wide range of applications, such as: PCR, AFLP, RFLP/PADP, Southern Blotting, Real-Time PCR.

Contents

Item	YGD100	YGD1000
RBC Lysis Buffer	360 ml	3500 ml
Cell Lysis Buffer	100 ml	1000 ml
Protein Removal Buffer	40 ml	400 ml
DNA Hydration Buffer*	50 ml	500 ml
RNase A (10 mg/ml) Solution	550 µl	5 ml

* DNA Hydration Buffer: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0.

Storage

HiYield™ Genomic DNA Isolation Kit (Blood) shall be shipped and stored dry at room temperature (15-25℃). With proper storage, HiYield™ Genomic DN A Isolation Kit (Blood) can be stored for up to 12 months without showing any deduction in performance and quality.

Quality Control

The quality of HiYield™ Genomic DNA Isolation Kit (Blood) is tested on a lot-to-lot basis by isolation of genomic DNA from 300 µl of fresh human whole blood. Purified DNA is quantified with a spectrophotometer and the yield of genomic DNA is 5-15 ug with A260/A280 ratio 1.8 - 2.0. The purified DNA is checked by electrophoresis.

Important Notes

Caution:

HiYield™ Genomic DNA Isolation Kit (Blood) contains irritants. During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.

DNA Hydration Buffer:

Using DNA Hydration Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. If using water instead of DNA Hydration Buffer, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. DNA in water should be stored at -20ºC to avoid degradation.

Yield and Quality of Purified DNA:

HiYield™ Genomic DNA Isolation Kit (Blood) is designed to purify high yields of high-quality DNA. The actual yield depends on the sample type, genome size of the source, and the quality of the starting material.

Reagents to Be Supplied by User:

1.5 ml microcentrifuge tubes, isopropanol, absolute ethanol for preparing 70% ethanol in ddH_2O.

Quick Reference Table

Experimental Conditions for Whole Blood:

Whole Blood Volume	300 µl	500 µl	1 ml	3 ml	10 ml
Number of white cells	2.1 x 10^6	3.5 x 10^6	7 x 10^6	2.1 x 10^7	7 x 10^7

Reagent Volume Used	Whole Blood Volume Used				
	300 µl	500 µl	1 ml	3 ml	10 ml
RBC Lysis Buffer	900 µl	1.5 ml	3 ml	9 ml	30 ml
Cell Lysis Buffer	300 µl	500 µl	1 ml	3 ml	10 ml
RNase A (10 mg/ml) Solution	1.5 µl	2.5 µl	5 µl	15 µl	50 µl
Protein Removal Buffer	100 µl	167 µl	333 µl	1 ml	3.33 ml
Isopropanol	300 µl	500 µl	1 ml	3 ml	10 ml
70% ethanol	300 µl	500 µl	1 ml	3 ml	10 ml
DNA Hydration Buffer	100 µl	100 µl	100 µl	300 µl	1 ml
Tube Size	1.5 ml	15 ml	15 ml	15 ml	50 ml

Experimental Conditions for Compromised Whole Blood:

Compromised Whole Blood Volume	3 ml	10 ml
Number of white cells	2.1 x 10^7	7 x 10^7

Reagent Volume Used	Compromised Whole Blood Volume Used	
	3 ml	10 ml
RBC Lysis Buffer	9 ml	30 ml
Cell Lysis Buffer	3 ml	10 ml
RNase A (10 mg/ml) Solution	15 µl	50 µl
Protein Removal Buffer	1.35 ml	4.5 ml
Isopropanol	4 ml	13.5 ml
70% ethanol	3 ml	10 ml
DNA Hydration Buffer	200 µl	500 µl
Tube Size	15 ml	50 ml

Protocol for 300 µl of Whole Blood

Please read the entire important notes prior to starting.

Step 1 Cell Lysis	Transfer 900 µl of RBC Lysis Buffer and 300 µl of whole blood into a 1.5 ml microcentrifuge tube then mix by inverting. Do not vortex. Incubate for 5 minutes at room temperature then centrifuge at 3,000 x g for 5 minutes to form a leukocyte (white blood cell) pellet. Carefully remove the supernatant, retaining approximately 50 µl of residual buffer and leukocyte pellet. Vortex the tube until the leukocyte pellet is completely resuspended in the residual buffer.
Step 2 Lysis	Add 300 µl of Cell Lysis Buffer to the tube then mix by vortex. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear and homogenous. During incubation, invert the tube every 3 minutes. Optional RNA Removal Step: Following 60ºC incubation, add 1.5 µl of RNase A (10 mg/ml) to the sample lysate then mix by vortex. Incubate at room temperature for 5 minutes.
Step 3 Protein Removal	Add 100 µl of Protein Removal Buffer to the sample lysate then vortex immediately for 10 seconds. Centrifuge at 14,000-16,000 x g for 3 minutes to form a tight, dark brown, protein pellet. NOTE: If the pellet is loose, incubate on ice for 5 minutes followed by centrifugation at 14,000-16,000 x g for another 3 minutes.
Step 4 DNA Precipitation	Transfer the supernatant to a clean 1.5 ml microcentrifuge tube then add 300 µl of isopropanol and mix well by gently inverting 20 times. Centrifuge at 14,000-16,000 x g for 5 minutes then carefully discard the supernatant and add 300 µl of 70% ethanol to wash the pellet. Centrifuge at 14,000-16,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.
Step 5 DNA Hydration	Add 100 µl of DNA Hydration Buffer then gently vortex for 10 seconds. Incubate at 60ºC for 5-10 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

Protocol for 3 ml of Whole Blood

Please read the entire important notes prior to starting.

Step 1 Cell Lysis	Transfer 9 ml of RBC Lysis Buffer and 3 ml of whole blood into a 15 ml centrifuge tube then mix by inverting. Do not vortex. Incubate for 5 minutes at room temperature then centrifuge at 3,000 x g for 5 minutes to form a leukocyte (white blood cell) pellet. Carefully remove the supernatant, retaining approximately 300 µl of residual buffer and leukocyte pellet. Vortex the tube until the leukocyte pellet is completely resuspended in the residual buffer.
Step 2 Lysis	Add 3 ml of Cell Lysis Buffer to the tube then mix by vortex. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear and homogenous. During incubation, invert the tube every 3 minutes. Optional RNA Removal Step: Following 60ºC incubation, add 15 µl of RNase A (10 mg/ml) to the sample lysate then mix by vortex. Incubate at room temperature for 10 minutes.
Step 3 Protein Removal	Add 1 ml of Protein Removal Buffer to the sample lysate then vortex immediately for 10 seconds. Centrifuge at 2,000-3,000 x g for 5 minutes to form a tight, dark brown, protein pellet. NOTE: If the pellet is loose, incubate on ice for 5 minutes followed by centrifugation at 3,000-6,000 x g for another 5 minutes.
Step 4 DNA Precipitation	Transfer the supernatant to a clean 15 ml centrifuge tube then add 3 ml of isopropanol and mix well by gently inverting 20 times. Centrifuge at 2,000-3,000 x g for 5 minutes then carefully discard the supernatant and add 3 ml of 70% ethanol to wash the pellet. Centrifuge at 2,000-3,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.
Step 5 DNA Hydration	Add 300 µl of DNA Hydration Buffer then gently vortex for 10 seconds. Incubate at 60ºC for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

Protocol for 10 ml of Whole Blood

Please read the entire important notes prior to starting.

Step 1 Cell Lysis	Transfer 30 ml of RBC Lysis Buffer and 10 ml of whole blood into a 50 ml centrifuge tube then mix by inverting. Do not vortex. Incubate for 5 minutes at room temperature then centrifuge at 3,000 x g for 5 minutes to form a leukocyte (white blood cell) pellet. Carefully remove the supernatant, retaining approximately 300 µl of residual buffer and leukocyte pellet. Vortex the tube until the leukocyte pellet is completely resuspended in the residual buffer.
Step 2 Lysis	Add 10 ml of Cell Lysis Buffer to the tube then mix by vortex. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear and homogenous. During incubation, invert the tube every 3 minutes. Optional RNA Removal Step: Following 60ºC incubation, add 50 µl of RNase A (10 mg/ml) to the sample lysate then mix by vortex. Incubate at room temperature for 10 minutes.
Step 3 Protein Removal	Add 3.33 ml of Protein Removal Buffer to the sample lysate then vortex immediately for 10 seconds. Centrifuge at 2,000-3,000 x g for 5 minutes to form a tight, dark brown, protein pellet. NOTE: If the pellet is loose, incubate on ice for 5 minutes followed by centrifugation at 3,000-6,000 x g for another 5 minutes.
Step 4 DNA Precipitation	Transfer the supernatant to a clean 50 ml centrifuge tube then add 10 ml of isopropanol and mix well by gently inverting 20 times. Centrifuge at 2,000-3,000 x g for 5 minutes then carefully discard the supernatant and add 10 ml of 70% ethanol to wash the pellet. Centrifuge at 2,000-3,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.
Step 5 DNA Hydration	Add 1 ml of DNA Hydration Buffer then gently vortex for 10 seconds. Incubate at 60ºC for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

Protocol for 10 ml of Compromised Whole Blood

Please read the entire important notes prior to starting.

Step 1 Cell Lysis	Transfer 30 ml of RBC Lysis Buffer and 10 ml of whole blood into a 50 ml centrifuge tube then mix by inverting. Do not vortex. Incubate for 5 minutes at room temperature then centrifuge at 3,000 x g for 5 minutes to form a leukocyte (white blood cell) pellet. Carefully remove the supernatant, retaining approximately 300 µl of residual buffer and leukocyte pellet. Vortex the tube until the leukocyte pellet is completely resuspended in the residual buffer.
Step 2 Lysis	Add 10 ml of Cell Lysis Buffer to the tube then mix by vortex. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear and homogenous. During incubation, invert the tube every 3 minutes. Optional RNA Removal Step: Following 60ºC incubation, add 50 µl of RNase A (10 mg/ml) to the sample lysate then mix by vortex. Incubate at room temperature for 10 minutes.
Step 3 Protein Removal	Add 4.5 ml of Protein Removal Buffer to the sample lysate then vortex immediately for 10 seconds. Centrifuge at 2,000-3,000 x g for 5 minutes to form a tight, dark brown, protein pellet. NOTE: If the pellet is loose, incubate on ice for 5 minutes followed by centrifugation at 3,000-6,000 x g for another 5 minutes.
Step 4 DNA Precipitation	Transfer the supernatant to a clean 50 ml centrifuge tube then add 13.5 ml of isopropanol and mix well by gently inverting 20 times. Centrifuge at 2,000-3,000 x g for 5 minutes then carefully discard the supernatant and add 10 ml of 70% ethanol to wash the pellet. Centrifuge at 2,000-3,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.
Step 5 DNA Hydration	Add 500 µl of DNA Hydration Buffer then gently vortex for 10 seconds. Incubate at 60ºC for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.