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HiYield™ Genomic DNA Isolation Kit (Cultured Cells)

Cat. No.:	YGU150	YGU1500
Product Name:	HiYield™ Genomic DNA Isolation Kit (Cultured Cells)	
Reactions:	150	1500
Sample:	Cultured animal cells	
Yield:	High yield and high quality DNA with A260/A280 = 1.8-2.0	
Elution Volume:	50 - 200 µl(varies according to starting sample amount)	
Format:	Reagent (scalable for a wide range of sample sizes)	
Operation:	Centrifuge	
Operation Time:	Within 60 minutes for 5 x 10^7 of cultured cells (including DNA rehydration)	

Description

HiYield™ Genomic DNA Isolation Kit (Cultured Cells) is designed specifically for isolating high molecular weight genomic, mitochondrial or viral DNA from large volumes of cultured animal cells in a scalable, simple-to-use format. Cultured animal cells are first treated with Cell Lysis Buffer which can efficiently lysing cultured cells. Protein is then removed from the lysate with the Protein Removal Buffer. The high-quality purified DNA can be utilized directly in 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 cultured animal cells within 60 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 direct use in DNA archiving, SNP analysis, PCR, southern blotting, real-time PCR, restriction digest, AFLP, RFLP, PADP.

Contents

Item	YGU150	YGU1500
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 (Cultured Cells) shall be shipped at room temperature (15-25℃). Cell Lysis Buffer, Protein Removal Buffer, DNA Hydration Buffer should be stored dry at room temperature (15-25ºC) for up to 2 years. RNase A should be stored at 4ºC for extended periods. With proper storage, HiYield™ Genomic DNA Isolation Kit (Cultured Cells) can be stored for up to 2 years without showing any deduction in performance and quality.

Quality Control

The quality of HiYield™ Genomic DNA Isolation Kit (Cultured Cells) is tested on a lot-to-lot basis by isolation of genomic DNA from 3 x 10^6 of cultured cells. Purified DNA is quantified with a spectrophotometer and the yield of genomic DNA is more than 5 ug with A260/A280 ratio 1.8 - 2.0. The purified DNA is checked by electrophoresis.

Important Notes

Caution:

HiYield™ Genomic DNA Isolation Kit (Cultured Cells) 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 (Cultured Cells) 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.

Quick Reference Table

Reagent Volume Used	Cultured Cells Volume Used		
	0.5-1 x 10^6	3-5 x 10^6	3-5 x 10^7
Cell Lysis Buffer	150 µl	600 µl	6 ml
RNase A (10 mg/ml) Solution	1 µl	3 µl	30 µl
Protein Removal Buffer	50 µl	200 µl	2 ml
Isopropanol	150 µl	600 µl	6 ml
70% ethanol	150 µl	600 µl	6 ml
DNA Hydration Buffer	50 µl	100 µl	200 µl
Tube Size	1.5 ml	1.5 ml	15 ml

Reagents to Be Supplied by User:

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

Protocol for 3-5 x 10^6 Cultured Cell Protocol

Please read the entire important notes prior to starting.

Step 1 Sample Preparation	**Adherent Cultured Animal Cells (trypsinize cells prior to harvesting)** Remove the culture medium and wash cells in PBS. Aspirate PBS and add 0.10-0.25% Trypsin in PBS. Once cells detach add the medium then transfer to a 1.5 ml microcentrifuge tube. Proceed with Suspension Cultured Animal cells. **Suspension Cultured Animal Cells** Transfer cells (3-5 x 10^6) to a 1.5 ml microcentrifuge tube then centrifuge for 5 minutes at 300 x g. Discard the supernatant retaining approximately 50 µl of residual buffer and cell pellet. Vortex the tube until the cell pellet is completely resuspended in the residual buffer.
Step 2 Lysis	Add 600 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 3 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 200 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 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 600 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 600 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 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

Protocol for 3-5 x 10^7 Cultured Cell Protocol

Please read the entire important notes prior to starting.

Step 1 Sample Preparation	**Adherent Cultured Animal Cells (trypsinize cells prior to harvesting)** Remove the culture medium and wash cells in PBS. Aspirate PBS and add 0.10-0.25% Trypsin in PBS. Once cells detach add the medium then transfer to a 15 ml microcentrifuge tube. Proceed with Suspension Cultured Animal cells. **Suspension Cultured Animal Cells** Transfer cells (3-5 x 10^7) to a 15 ml microcentrifuge tube then centrifuge for 5 minutes at 300 x g. Discard the supernatant retaining approximately 50 µl of residual buffer and cell pellet. Vortex the tube until the cell pellet is completely resuspended in the residual buffer.
Step 2 Lysis	Add 6 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 30 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 2 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 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 3 minutes.
Step 4 DNA Precipitation	Transfer the supernatant to a clean 15 ml microcentrifuge tube then add 6 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 6 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 200 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.