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HiYield™ Genomic DNA Isolation Kit (Tissue)

Cat. No.:	YGZ150	YGZ1500
Product Name:	HiYield™ Genomic DNA Isolation Kit (Tissue)	
Reactions:	150	1500
Sample:	Scalable tissue samples	
Yield:	High yield and high quality DNA with A260/A280 = 1.8-2.0	
Elution Volume:	100-200 µl	
Format:	Reagent (scalable for a wide range of sample sizes)	
Operation:	Centrifuge	
Operation Time:	Within 80 minutes for 200 mg of tissues (including DNA rehydration)	

Description

HiYield™ Genomic DNA Isolation Kit (Tissue) is designed specifically for isolating high molecular weight genomic, mitochondrial or viral DNA from large volumes of tissue samples in a scalable, simple-to-use format. Tissues are first treated with Cell Lysis Buffer and then treated with Proteinase K which can efficiently lyse tissues. Protein is then removed from the lysate with the Protein Removal Buffer. The archive-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 archive-quality DNA from tissues.
A complete solution from sample purification to storage.
Complete removal of all contaminants for reliable downstream applications.

Applications

Purified DNA is ready for direct use in DNA archiving, PCR, restriction digest, southern blotting, real-time PCR, AFLP, RFLP, PADP...etc.

Quality Control

The quality of HiYield™ Genomic DNA Isolation Kit (Tissue) is tested on a lot-to-lot basis by isolation of genomic DNA from 10 mg of tissues. Purified DNA is quantified with a spectrophotometer and the yield of genomic DNA is more than 5 µg with A260/A280 ratio 1.8 - 2.0. The purified DNA is checked by electrophoresis.

Contents

Item	YGZ150	YGZ1500
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
Proteinase K**	22 mg	206 mg

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

** Add ddH_2O to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely disolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down.

Storage

Cell Lysis Buffer, Protein Removal Buffer and DNA Hydration Buffer should be stored dry at room temperature (15-25ºC) for up to 2 years. Proteinase K and RNase A should be stored at 4ºC for extended periods. After the ddH_2O is added, the Proteinase K mixture should be stored at 4ºC.

Important Notes

Caution:

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

Reagents to Be Supplied by User:

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

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 (Tissue) 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.

Reagent volume required for different tissue weight:

Tissue weight	1-5 mg	10-20 mg	150-200 mg
Tube size	1.5 ml	1.5 ml	15 ml
Cell Lysis Buffer	100 µl	600 µl	6 ml
Proteinase K (ddH2O added)	3 µl	12 µl	120 µl
RNase A (10 mg/ml)	1 µl	3 µl	30 µl
Protein Removal Buffer	33 µl	200 µl	2 ml
Isopropanol	100 µl	600 µl	6 ml
70% ethanol	100 µl	600 µl	6 ml
DNA Hydration Buffer	100 µl	100 µl	200 µl

Protocol for 10-20 mg Tissue

Please read the entire important notes prior to starting.

Step 1 Tissue Dissociation	Transfer 10-20 mg of tissue (0.5 cm of mouse tail) to a 1.5 ml microcentrifuge tube and use a micropestle to grind the tissue a few times. Add 600 µl of Cell Lysis Buffer to the tube and continue to homogenize the sample tissue with grinding.
Step 2 Lysis	Add 12 µl of Proteinase K to the tube then mix by vortex. Incubate at 60ºC for 30-60 minutes or until the tissue has dissolved completely. During incubation, invert the tube periodically. 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 150-200 mg Tissue

Please read the entire important notes prior to starting.

Step 1 Tissue Dissociation	Freeze 150-200 mg of tissue with liquid nitrogen then grind to a fine powder using a mortar and pestle. Add 6 ml of Cell Lysis Buffer to the morter and continue to homogenize the sample tissue with grinding. Then transfer the homogenized sample to a 15 ml centrifuge tube.
Step 2 Lysis	Add 120 µl of Proteinase K to the tube then mix by vortex. Incubate at 60ºC for 30-60 minutes or until the tissue has dissolved completely. During incubation, invert the tube periodically. 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 10 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 5 minutes.
Step 4 DNA Precipitation	Transfer the supernatant to a clean 15 ml centrifuge 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.