www.real-biotech.com
Real Biotech Corporation
13F.-2, No.33, Sec. 1, Minsheng Rd., Banqiao City, Taipei County 220, Taiwan, R. O. C.
Tel: +886 2 2950 9000 Fax: +886 2 2950 0505

HiYield Genomic DNA Isolation Kit (Yeast)

Cat. No.:	YGY100	YGY300
Product Name:	HiYield Genomic DNA Isolation Kit (Yeast)	
Reactions:	100	300
Sample:	Up to 2 x 10^8 of yeast and a variety of fungus species	
Yield:	Up to 14.5ug for 2 x 10^8 of Saccharomyces cerevisiae	
Elution Volume:	50-100 µl	
Format:	Reagent	
Operation:	Centrifuge	
Operation Time:	Within 40 Minutes	

Description

HiYield Genomic DNA Isolation Kit (Yeast) is ideal for purification of high-molecular-weight genomic, mitochondrial or viral DNA from Saccharomyces cerevisiae and a variety of other yeast and fungus species in a simple and gentle reagent DNA precipitation method. The convenient, scalable purification system removes contaminants and enzyme inhibitors such as proteins and divalent cations. Purified DNA (A260/A280=1.8-2.0) is ready for immediate use in sensitive downstream applications or for archiving.

Features

1. Convenient, scalable purification procedure.
2. Reproducible recoveries for constant results.
3. Complete removal of all contaminants for sensitive downstream applications.

Contents

Item	YGY100	YGY300
Cell Suspension Buffer	90 ml	225 ml
Cell Lysis Buffer	40 ml	100 ml
Protein Removal Buffer	15 ml	40 ml
DNA Hydration Buffer*	50 ml	50 ml

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

Storage

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

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.

Quality Control

The quality of HiYield Genomic DNA Isolation Kit (Yeast) is tested on a lot-to-lot basis by isolation of Genomic DNA from 2 x 10^8 of Saccharomyces cerevisiae. A 15 µl aliquot of purified genomic DNA from a 100 µl eluate is analyzed by electrophoresis on a 1% agarose gel. Genomic DNA is quantified with a spectrophotometer and the yield of Genomic DNA is more than 10 µg.

Important Notes

Caution:

HiYield Genomic DNA Isolation Kit (Yeast) 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, β-mercaptoethanol, zymolase or lyticase, RNase A (50 mg/ml), 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.

Yeast Double-stranded RNA Killer Virus:

Yeast Double-stranded RNA Killer Virus can be found in the majority of S. cerevisiae laboratory strains. The ds RNA Killer Virus produces a ~5000 bp band, sometimes an extremely faint ~2000 bp band, on an agarose gel. These bands do not interfere with most downstream applications, excluding exact quantitation of the sample. The ds RNA Killer Virus can be cured by decreasing the suggested amount of isopropanol addition by ½ in DNA Precipitation step; however, this will also result in reduced genomic DNA yield.

Yield and Quality of Purified DNA:

HiYield Genomic DNA Isolation Kit (Yeast) 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.

Protocol

Please read the entire important notes prior to starting.

Step 1 Cell Harvesting	1. Transfer fungus cells (up to 2 x 10^8) to a 1.5 ml microcentrifuge tube. 2. Harvest fungus cells by centrifugation for 10 minutes at 5,000 x g. 3. Discard the supernatant and resuspend the pellet in **600 µl of Cell Suspension Buffer**. 4. Add 2 µl of ß-mercaptoethanol and 200 U of lyticase or zymolase. Then incubate at 30ºC for 30 minutes. 5. Centrifuge the mixture for 10 minutes at 2,000 x g to harvest the spheroplast and then remove the supernatant.
Step 2 Cell Lysis	1. Add **300 µl of Cell Lysis Buffer** and resuspend the cell pellet by pipette. 2. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear. During incubation, invert the tube every 3 minutes. Optional RNA Removal Step: Following 60ºC incubation, add 5 µl of RNase A (50 mg/ml) to the clear sample lysate and mix by vortex. Incubate at room temperature for 10 minutes.
Step 3 Protein Removal	1. Add **100 µl of Protein Removal Buffer** to the sample lysate and vortex IMMEDIATELY for 10 seconds. 2. Centrifuge at 14,000 -16,000 x g for 3 minutes to form a tight and white protein pellet. If the pellet is not tight enough, incubate on ice for 5 minutes followed by centrifugation at 14,000 -16,000 x g for another 3 minutes.
Step 4 DNA Precipitation	1. Being careful not to draw any of the protein pellet into the pipette, transfer the supernatant from Step 3 to a new 1.5 ml microcentrifuge tube. 2. Add 300 µl of isopropanol and mix well by gently inverting 20 times. 3. Centrifuge at 14,000 -16,000 x g for 5 minutes. 4. Carefully remove the supernatant and add 300 µl of 70% ethanol to wash the pellet. 5. Centrifuge at 14,000 -16,000 x g for 3 minutes. 6. Discard the supernatant and then air-dry the pellet for 10 minutes. (DO NOT dry the DNA pellet with by vacuum centrifuge and avoid over drying the DNA pellet.)
Step 5 DNA Hydration	1. Add **50-100 µl of DNA Hydration Buffer** or ddH_2O and then incubate at 60ºC for 10 minutes to dissolve the DNA pellet. (Occasionally tapping the bottom of the tube during incubation will promote DNA rehydration.)