Geneaid

Instruction Manual
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For Research Use Only

Geneaid™ DNA Isolation Kit (Yeast)

GEY100, GEY300

Advantages

Sample: up to 2×10^8 yeast and other fungus species
Yield: high yield, high quality DNA (A260/A280 = 1.8-2.0)
Format: scalable DNA precipitation method
Kit Storage: dry at room temperature (15-25°C)

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Introduction

The Geneaid™ DNA Isolation Kit (Yeast) offers a simple and gentle reagent DNA precipitation method for isolating high molecular weight genomic, mitochondrial or viral DNA from *Saccharomyces cerevisiae* and a variety of other yeast and fungus species. This highly versatile solution based system offers a convenient procedure with minimal hands on time. The provided Sorbitol Buffer, when combined with zymolase or lyticase, will efficiently lyse yeast and other fungus species cell walls consisting of chitin and polysaccharides. The extracted DNA (A260/A280 = 1.8-2.0), is suitable for use in PCR or other enzymatic reactions.

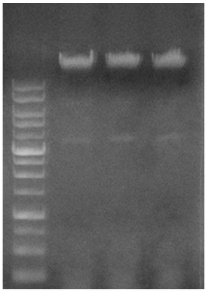
Quality Control

The Geneaid™ DNA Isolation Kit (Yeast) is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system. Genomic DNA is isolated from *S. cerevisiae* (2×10^8). A 15 µl aliquot of purified genomic DNA from a 100 µl eluate is analyzed by electrophoresis on a 1% agarose gel.

Components and Storage

Item	Volume	Product	Shipping	Storage
Sorbitol Buffer	4.5 ml	GEY004	room temperature	dry at room temperature (15-25ºC)
	90 ml	GEY100		
	225 ml	GEY300		
Cell Lysis Buffer	3 ml	GEY004	room temperature	dry at room temperature (15-25ºC)
	40 ml	GEY100		
	100 ml	GEY300		
Protein Removal Buffer	1 ml	GEY004	room temperature	dry at room temperature (15-25ºC)
	15 ml	GEY100		
	40 ml	GEY300		
DNA Hydration Buffer (10 mM Tris-HCl, 1 mM EDTA, pH8.0)	1 ml	GEY004	room temperature	dry at room temperature (15-25ºC)
	50 ml	GEY100		
	50 ml	GEY300		

Geneaid DNA Isolation Kit (Yeast) Functional Test Data



M 1 2 3

Figure 1. Genomic DNA (approx. 30 kb) was extracted using the Geneaid DNA Isolation Kit (Yeast). *S. cerevisiae* (2×10^8) was harvested by centrifugation at 5,000 x g for 10 minutes. A 15 µl aliquot of extracted genomic DNA from a 100 µl eluate was analyzed by electrophoresis on a 1% agarose gel.
M = 1 Kb DNA Ladder

Test	DNA Concentration	260/280	260/230	Yield
1	115.5 µg/ml	1.91	1.87	11.6 µg
2	142.9 µg/ml	1.92	2.01	14.3 µg
3	137.1 µg/ml	1.91	1.97	13.7 µg

Geneaid DNA Isolation Kit (Yeast) Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.

Additional Requirements

1.5 ml microcentrifuge tubes, zymolase or lyticase, RNase A (50 mg/ml), isopropanol, absolute ethanol for preparing 70% ethanol in ddH_2O

Caution

During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.

1. Cell Harvesting

A. Yeast/Fungus on Agar Plate

Use an inoculating loop to transfer 50-200 mg of yeast/fungus colonies (up to 2 x 10^8) from an agar plate to a 1.5 ml microcentrifuge tube containing **600 µl of Sorbitol Buffer**.

B. Yeast/Fungus in Broth

Transfer yeast/fungus cells in broth to a 1.5 ml microcentrifuge tube. Centrifuge for 10 minutes at 5,000 x g then discard the supernatant. Weigh 50-200 mg of wet pellet (up to 2 x 10^8) for DNA extraction. Repeat to harvest yeast/fungus cells by centrifugation using the same microcentrifuge tube if required. Re-suspend the cells in **600 µl of Sorbitol Buffer**.

2. Lysis

Add **200 U of Lyticase or Zymolase** then mix well. Incubate at 30ºC for 30 minutes. Centrifuge for 10 minutes at 2,000 x g to form a spheroplast pellet. Discard the supernatant then add **300 µl of Cell Lysis Buffer** then resuspend the cell pellet by pipette. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear.

NOTE: 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 then mix by vortex. Incubate at room temperature for 10 minutes.

3. Protein Removal

Add **100 µl of Protein Removal Buffer** to the sample lysate then vortex IMMEDIATELY for 10 seconds. Centrifuge at 14-16,000 x g for 3 minutes to form a tight, white, protein pellet.

NOTE: Following centrifugation the protein should form a tight, white, pellet. If the pellet is not tight then incubate on ice for 5 minutes followed by centrifugation at 14-16,000 x g for another 3 minutes.

4. DNA Precipitation

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. Add **300 µl of isopropanol** and mix well by gently inverting 20 times. Centrifuge at 14-16,000 x g for 5 minutes. Carefully remove the supernatant then add **300 µl of 70% ethanol** to wash the pellet. Centrifuge at 14-16,000 x g for 3 minutes. Discard the supernatant then air-dry the pellet for 10 minutes.

NOTE: DO NOT dry the DNA pellet with vacuum centrifuge and avoid over drying the DNA pellet.

5. DNA Rehydration

Add **50-100 µl of DNA Hydration Buffer** or ddH_2O then incubate at 60ºC for 10 minutes to dissolve the DNA pellet.

NOTE: Occasionaly tapping the bottom of the tube during incubation will promote DNA rehydration. 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.

Troubleshooting

Problem	Cause	Solution
Low Yield	A. Sample lysis or homogenization was incomplete B. Incorrect DNA precipitation C. Precipitate was formed during Step 4	A. Starting material should be reduced B. Following isopropanol addition, increase standing time to improve DNA precipitation. Following centrifugation, carefully remove the supernatant without contacting the DNA pellet. C. Reduce starting material
Degraded DNA	A. Incorrect sample preparation B. Incorrect sample storage	A. Process samples immediately after collection B. Extracted DNA should be stored at -20ºC
RNA Contamination	A. Did not perform optional RNase A treatment	A. If DNA is used for sensitive downstream applications it might be necessary to extract RNA-free DNA. Therefore, RNase A treatment should be performed
Eluted DNA does not perform well in downstream applications	A. Residual ethanol contamination	A. Increase DNA pellet drying time to ensure residual ethanol is completely evaporated

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