

Geneaid™ DNA Isolation Kit (Yeast) Quick Protocol

For research use only

Catalogue Number

GEY004, GEY100, GEY300

Instruction Manual Download

When using this product for the first time, or if you are unfamiliar with the procedure, please scan the QR code and download the complete instruction manual.

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Additional Requirements

1.5 ml microcentrifuge tubes, zymolase or lyticase, RNase A (50 mg/ml), isopropanol, absolute ethanol for preparing 70% ethanol in ddH₂O

Yeast and other Fungus Species Protocol Procedure

1. Cell Harvesting

A. Yeast/Fungus on Agar Plate

Use an inoculating loop to transfer **50-200 mg of yeast/fungus colonies (up to 2×10^8)** 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 2×10^8)** 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 30°C for 30 minutes. Centrifuge for 10 minutes at 2,000 x g to form a spheroplast pellet. Discard the supernatant. Add **300 µl of Cell Lysis Buffer** then resuspend the cell pellet by pipette. Incubate at 60°C 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 60°C incubation, add 5 µl of RNase A (50 mg/ml) to the clear sample lysate then mix by vortex.

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 an additional 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 ddH₂O. Incubate at 60°C for 10 minutes to dissolve the DNA pellet. NOTE: Occasionally 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. ddH₂O should be fresh as ambient CO₂ can quickly cause acidification. DNA in water should be stored at -20°C to avoid degradation.

Kit Components And Storage

Item	Volume	Product	Shipping	Storage
Sorbitol Buffer	4.5 ml	GEY004	room temperature	dry at room temperature (15-25°C)
	90 ml	GEY100		
	225 ml	GEY300		
Cell Lysis Buffer	3 ml	GEY004	room temperature	dry at room temperature (15-25°C)
	40 ml	GEY100		
	100 ml	GEY300		
Protein Removal Buffer	1 ml	GEY004	room temperature	dry at room temperature (15-25°C)
	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 (15-25°C)
	50 ml	GEY100		
	50 ml	GEY300		

Geneaid™ DNA Isolation Kit (Yeast) Functional Test Data

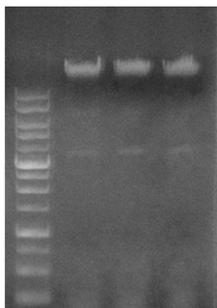


Figure 1. Genomic DNA (approx. 30 kb) was extracted using the Geneaid™ DNA isolation Kit (Yeast). *Saccharomyces cerevisiae* (2×10^8) was harvested by centrifugation at 5,000 x g for 10 minutes. A 15 µl aliquot of extracted genomic DNA from a 100 µl eluate was analyzed by electrophoresis on a 1% agarose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

M = Geneaid 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

M 1 2 3

Troubleshooting

Problem	Cause	Solution
Low Yield	<ul style="list-style-type: none"> A. Sample lysis or homogenization was incomplete B. Incorrect DNA precipitation C. Precipitate was formed during Step 4 	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> A. Incorrect sample preparation B. Incorrect sample storage 	<ul style="list-style-type: none"> A. Process samples immediately after collection B. Extracted DNA should be stored at -20°C
RNA Contamination	<ul style="list-style-type: none"> A. Did not perform optional RNase A treatment 	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> A. Residual ethanol contamination 	<ul style="list-style-type: none"> A. Increase DNA pellet drying time to ensure residual ethanol is completely evaporated