

# Geneaid™ DNA Isolation Kit (Yeast)

For research use only

Store at Room Temperature

Catalogue Numbers

GEY100

GEY300

Geneaid



CERTIFICATE NO. QAIC/TW/50077  
ISO 9001:2008 QMS

## 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 *Saccharomyces cerevisiae* ( $2 \times 10^8$ ). A 15 µl aliquot of purified genomic DNA from a 100 µl eluate is analyzed by electrophoresis on a 1% agarose gel.

## Advantages

- High molecular weight genomic DNA extraction from a variety of yeast/fungus samples using a simple and gentle DNA precipitation method
- Time: 40 minutes
- Sample: up to  $2 \times 10^8$  yeast and other fungus species
- Convenient: includes premixed Sorbitol Buffer
- Format: DNA precipitation reagent system
- Elution volume: 50-100 µl
- Cost effective

## Applications

PCR, AFLP, RFLP/PADP, Southern Blotting, Real-time PCR

## Caution

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

## Additional Requirements

1.5 ml microcentrifuge tubes, zymolase or lyticase, RNase A (50 mg/ml), isopropanol, absolute ethanol for preparing 70% ethanol in ddH<sub>2</sub>O

## 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		

# Yeast and other Fungus Species Protocol Procedure

Please read the entire instruction manual prior to starting the 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  $2 \times 10^8$ )** from an agar plate to a 1.5 ml microcentrifuge tube.

### 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  $2 \times 10^8$ )** for DNA extraction. Repeat to harvest yeast/fungus cells by centrifugation using the same microcentrifuge tube if required.

## 2. Lysis

1. Add **300  $\mu$ l of Cell Lysis Buffer** then resuspend the cell pellet by pipette.
2. Incubate at 60°C 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 60°C incubation, add 5  $\mu$ l 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

1. Add **100  $\mu$ l of Protein Removal Buffer to the sample lysate** then vortex **IMMEDIATELY** for 10 seconds.
2. 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

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

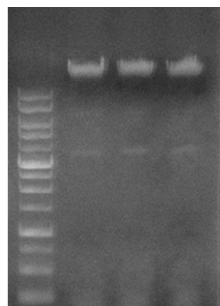
NOTE: DO NOT dry the DNA pellet with vacuum centrifuge and avoid over drying the DNA pellet.

## 5. DNA Rehydration

1. Add **50-100  $\mu$ l of DNA Hydration Buffer** or ddH<sub>2</sub>O then 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. ddH<sub>2</sub>O should be fresh as ambient CO<sub>2</sub> can quickly cause acidification. DNA in water should be stored at -20°C to avoid degradation.

## 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* ( $2 \times 10^8$ ) was harvested by centrifugation at 5,000 x g for 10 minutes. A 15  $\mu$ l aliquot of extracted genomic DNA from a 100  $\mu$ l 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 $\mu$ g/ml	1.91	1.87	11.6 $\mu$ g
2	142.9 $\mu$ g/ml	1.92	2.01	14.3 $\mu$ g
3	137.1 $\mu$ g/ml	1.91	1.97	13.7 $\mu$ g

M 1 2 3

## 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 -20°C
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

## Related DNA/RNA Extraction Products

Plasmid DNA Purification		
Product	Package Size	Catalogue Number
Presto™ Mini Plasmid Kit	100/300 preps	PDH100/300
Presto™ Midi Plasmid Kit	25 preps	PIF025
Presto™ Midi Plasmid Kit (Endotoxin Free)	25 preps	PIFE25
Large Plasmid DNA Extraction Kit	100/300 preps	PDL100/300
High-Speed Plasmid Advance Kit (50-100 ml)	25 preps	PA025
Geneaid™ Plasmid Mini Kit	40/100 preps	PAE040/100
Geneaid™ Plasmid Midi Kit	25 preps	PI025
Geneaid™ Plasmid Midi Kit (Endotoxin Free)	25 preps	PIE25
Presto™ Plasmid DNA Concentration Kit	250/500/1000 preps	PC0250/500/1000
Geneaid™ Plasmid Maxi Kit	10/25 preps	PM010/25
Geneaid™ Plasmid Maxi Kit (Endotoxin Free)	10/25 preps	PME10/25
Presto™ 96 Well Plasmid Kit	4/10 x 96 preps	96PDV04/10, 96PDC04/10
Presto™ Plasmid 96 Well Binding Plate	10 plates	96BP01
Presto™ Plasmid 96 Well Filter Plate	10 plates	96PFP01
Post Reaction DNA Purification		
Product	Package Size	Catalogue Number
GenepHlow™ Gel Extraction Kit	100/300 preps	DFG100/300
GenepHlow™ PCR Cleanup Kit	100/300 preps	DFC100/300
GenepHlow™ Gel/PCR Kit	100/300 preps	DFH100/300
GenepHlow™ DNA Cleanup Maxi Kit	10/25 preps	DFM010/025
Small DNA Fragments Extraction Kit	100/300 preps	DF101/301
Large DNA Fragments Extraction Kit	100/300 preps	DFL100/300
Presto™ 96 Well PCR Cleanup Kit	4/10 x 96 preps	96DFH04/10
Presto™ 96 Well Gel Extraction Kit	4/10 x 96 preps	96DFG04/10
Presto™ PCR Cleanup Kit 96 Well Binding Plate	10 plates	96DBP01
DNA Cleanup Kit	100/300 preps	DP100/300
G-25 Gel Filtration Desalting Column	50 rxns	CG025
G-50 Gel Filtration Dye Terminator Removal Column	50 rxns	CG050
96-Well G-50 Gel Filtration Plate	4/10 x 96 rxns	CGP04/10
Gel Extraction Tool	25 pcs	GXT025
Virus DNA/RNA Purification		
Product	Package Size	Catalogue Number
Plant Virus RNA Kit	50/100 preps	PVR050/100
Viral Nucleic Acid Extraction Kit II	50/100/300 preps	VR050/100/300
Viral Nucleic Acid Extraction Kit III	50/100/300 preps	VI050/100/300

## Related DNA/RNA Extraction Products

Total RNA Purification		
Product	Package Size	Catalogue Number
Total RNA Mini Kit (Blood/Cultured Cell)	50/100/300 preps	RB050/100/300
Total RNA Mini Kit (Tissue)	50/100/300 preps	RT050/100/300
Total RNA Mini Kit (Plant)	50/100/300 preps	RP050/100/300
Presto™ Mini RNA Bacteria Kit	50/100/300 preps	RBB050/100/300
Presto™ Mini RNA Yeast Kit	50/100/300 preps	RBY050/100/300
miRNA Isolation Kit	50/100 preps	RMI050/100
GENEzol™ Reagent	100/200 rxns	GZR050/100/200
GENEzol™ TriRNA Bacteria Kit	50/100 rxns	GZB050/100
GENEzol™ TriRNA Pure Kit	50/100/200 rxns	GZX050/100/200, GZX051/101/201
TriRNA Pure Kit	50/100/200 preps	TRP050/100/200
RNA Cleanup Kit	50/100 preps	PR050/100
Genomic DNA Purification		
Product	Package Size	Catalogue Number
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	GB100/300
Genomic DNA Midi Kit (Blood/Cultured Cell)	25 preps	GDI25
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	GDM10/25
Genomic DNA Mini Kit (Tissue)	50/100/300 preps	GT050/100/300
gSYNC™ DNA Extraction Kit	100/300 preps	GS100/300
Genomic DNA Mini Kit (Plant)	100 preps	GP100
Genomic DNA Maxi Kit (Plant)	10/25 preps	GPM10/25
Geneaid™ DNA Isolation Kit (Blood)	100/1,000 rxns	GEB100/01K(+)
Geneaid™ DNA Isolation Kit (Bacteria)	150/1,500 rxns	GEE150/1.5K(+)
Geneaid™ DNA Isolation Kit (Tissue)	150/1,500 rxns	GET150/1.5K(+)
Geneaid™ DNA Isolation Kit (Cultured Cell)	150/1,500 rxns	GEC150/1.5K(+)
GENEzol™ DNA Reagent Plant	100/200 rxns	GR100/200
Presto™ Mini gDNA Yeast Kit	100/300 preps	GBYB100/300
Presto™ Mini gDNA Bacteria Kit	100/300 preps	GBB100/101/300/301
Geneius™ Micro DNA Extraction Kit	100/300 preps	GMB100/300
Presto™ Buccal Swab gDNA Extraction Kit	100/300 preps	GSK100/300
Presto™ 96 Well Blood Genomic DNA Extraction Kit	4/10 x 96 preps	96GBP04/10
Magnetic Beads DNA/RNA Purification		
Product	Package Size	Catalogue Number
Magnetic Beads Genomic DNA Kit (Bacteria)	48/96 rxns	ME048/96
Magnetic Beads Genomic DNA Kit (Blood)	48/96 rxns	MB048/96
Magnetic Beads Micro gDNA Kit	48/96 rxns	MM048/96
Magnetic Beads Genomic DNA Kit (Plant)	48/96 rxns	MP048/96
Magnetic Beads Viral DNA/RNA Kit	48/96 rxns	MV048/96
Magnetic Beads PCR Cleanup Kit	48/96 rxns	MC048/96

For additional product information, please visit [www.geneaid.com](http://www.geneaid.com). Thank you!