gYEAST Genomic DNA Kit

For research use only

Catalogue Numbers

IB47265
IB47266
IB47267

Introduction

The gYEAST Genomic DNA Kit 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 β-mercaptoethanol, 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 gYEAST Genomic DNA Kit is tested on a lot-to-lot basis. *Saccharomyces cerevisiae* (2×10^8) is harvested by centrifugation at 5,000 x g for 10 minutes. 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
- Sample: up to 2×10^8 yeast and other fungus species
- Convenient: includes premixed Sorbitol Buffer
- Format: DNA precipitation reagent system
- Elution volume: 50-100 µl

Applications

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

Caution

The gYEAST Genomic DNA Kit 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, β-mercaptoethanol, zymolase or lyticase, RNase A (50 mg/ml), isopropanol, absolute ethanol for preparing 70% ethanol in ddH_2O

Components and Storage

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.

Item	Volume	Product	Shipping	Storage
Sorbitol Buffer	4.5 ml	IB47265	room temperature	dry at room temperature (15-25ºC) for up to 1 year
	90 ml	IB47266		
	225 ml	IB47267		
Cell Lysis Buffer	3 ml	IB47265	room temperature	dry at room temperature (15-25ºC) for up to 1 year
	40 ml	IB47266		
	100 ml	IB47267		
Protein Removal Buffer	1 ml	IB47265	room temperature	dry at room temperature (15-25ºC) for up to 1 year
	15 ml	IB47266		
	40 ml	IB47267		
DNA Hydration Buffer (10 mM Tris-HCl, 1 mM EDTA, pH8.0)	1 ml	IB47265	room temperature	dry at room temperature (15-25ºC) for up to 1 year
	50 ml	IB47266		
	50 ml	IB47267		

Yeast and other Fungus Species Protocol Procedure

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

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 then resuspend the pellet in **600 µl of Sorbitol 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 then remove the supernatant.

2. Lysis

1. **Add 300 µl of Cell Lysis Buffer** then resuspend the cell pellet by pipette.
2. 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

1. **Add 100 µl 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 µl 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 µl 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 by vacuum centrifuge and avoid over drying the DNA pellet.

5. DNA Rehydration

1. **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.

gYEAST Genomic DNA Kit Functional Test Data

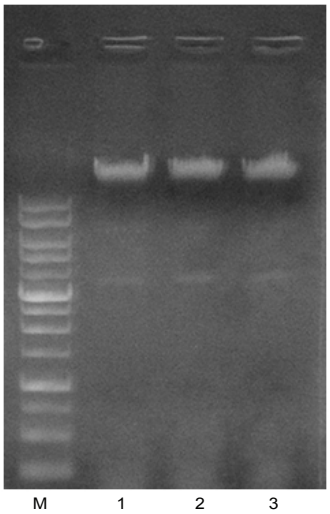

Figure 1. Genomic DNA (approx. 30 kb) was extracted using the gYEAST Genomic DNA Kit. *Saccharomyces cerevisiae* (2×10^8) was harvested by centrifugation at 5,000 x g for 10 minutes. A 15 µl aliquot of extracted genomic DNA from a 100 µl eluate was analyzed by electrophoresis on a 1% agarose gel.

M = 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

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

Related DNA/RNA Purification and Extraction Products

Plasmid DNA Purification		
Product	Package Size	Catalogue Number
I-Blue Mini Plasmid Kit	100/300 preps	IB47171/172
I-Blue Midi Plasmid Kit	25 preps	IB47181
I-Blue Midi Plasmid Kit (Endotoxin Free)	25 preps	IB47191
Fast Ion Plasmid Midi Kit	25 preps	IB47111
Fast Ion Plasmid Midi Kit (Endotoxin Free)	25 preps	IB47113
Fast Ion Plasmid Maxi Kit	10/25 preps	IB47121/122
Fast Ion Plasmid Maxi Kit (Endotoxin Free)	10/25 preps	IB47124/125
96-Well Plasmid Kit	4/10 x 96 preps	IB47151/152
Post Reaction DNA Purification		
Product	Package Size	Catalogue Number
Gel/PCR DNA Fragments Extraction Kit	100/300 preps	IB47020/030
Small DNA Fragments Extraction Kit	100/300 preps	IB47061/062
96-Well Gel/PCR DNA Extraction Kit	4/10 x 96 preps	IB47040/050
Genomic DNA Extraction and Purification		
Product	Package Size	Catalogue Number
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	IB47201/202
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10 preps	IB47210
Genomic DNA Mini Kit (Tissue)	50/300 preps	IB47221/222
gMax Mini Kit (Blood/Tissue)	100/300 preps	IB47281/282
Genomic DNA Mini Kit (Plant)	100 preps	IB47230
Genomic DNA Maxi Kit (Plant)	10/25 preps	IB47240/241
gBAC Mini DNA Bacteria Kit	100/300 preps	IB47291/292
gYEAST Genomic DNA Kit	100/300 preps	IB47266/267
96-Well Genomic DNA Extraction Kit	4/10 x 96 preps	IB47251/252
96-Well Genomic DNA Extraction Kit (Plant)	4/10 x 96 preps	IB47271/272

Related DNA/RNA Purification and Extraction Products

RNA Extraction and Purification		
Product	Package Size	Catalogue Number
Total RNA Mini Kit (Blood/Cultured Cell)	50/100/300 preps	IB47321/322/323
Total RNA Maxi Kit (Blood/Cultured Cell)	10 preps	IB47330
Total RNA Mini Kit (Tissue)	50/100 preps	IB47301/302
Total RNA Maxi Kit (Tissue)	10 preps	IB47310
Total RNA Mini Kit (Plant)	50/100 preps	IB47341/342
Total RNA Maxi Kit (Plant)	10 preps	IB47350
rBAC Mini RNA Bacteria Kit	100/300 preps	IB47421/412
rYeast Total RNA Mini Kit	50/100/300 preps	IB47411/422
96-Well Total RNA Extraction Kit (Plant)	4/10 x 96 preps	IB47381/382
96-Well Total RNA Extraction Kit	4/10 x 96 preps	IB47360/361
miRNA Isolation Kit	100 preps	IB47371
Virus DNA/RNA Purification		
Product	Package Size	Catalogue Number
Viral Nucleic Acid Extraction Kit II	50/100/300 preps	IB47401/402/403

For additional product information, please visit www.ibisci.com. Thank you!