Geneaid

Instruction Manual
Ver. 04.27.17
For Research Use Only

Presto™ Mini gDNA Yeast Kit

GBYB004 (4 Preparation Sample Kit)
GBYB100 (100 Preparation Kit)
GBYB300 (300 Preparation Kit)

Advantages

Sample: up to 2 x10^8 yeast and other fungus cells
gDNA Yield: up to 10 µg of genomic DNA from 2 x10^8 *Saccharomyces cerevisiae*
Format: beadbeating tubes and spin columns
Time: within 40 minutes
Elution Volume: 30-200 µl
Kit Storage: dry at room temperature (15-25°C)

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Introduction

The Presto™ Mini gDNA Yeast Kit was designed for rapid isolation of genomic DNA from cultured yeast and fungus. The yeast/fungus cells are directly added into beadbeating tubes containing ceramic beads and lysis buffer. Samples are efficiently lysed without the use of degestive enzymes (such as Lyticase or Zymolase) using a bead beating instrument or a standard vortex. The cell lysate is then mixed with Binding Buffer and the genomic DNA is bound by the GD Column. The column is then washed and the DNA is eluted with Elution Buffer. DNA phenol extraction or alcohol precipitation is not required and the entire procedure can be completed within 40 minutes. The purified genomic DNA is ready for use in PCR, restriction enzyme digestion, and sequencing reactions.

Quality Control

The quality of the Presto™ Mini gDNA Yeast Kit is tested on a lot-to-lot basis by isolating genomic DNA from 2 x10^8 *Saccharomyces cerevisiae* cells. Following the purification process, greater than 8 µg of genomic DNA is obtained and the A260/A280 ratio is between 1.7-2.0. The purified genomic DNA is analyzed by electrophoresis.

Kit Components

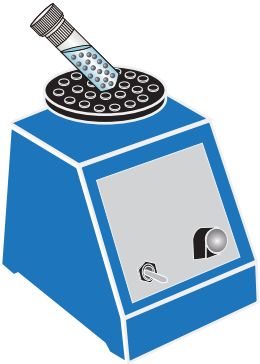
Component	GBYB004	GBYB100	GBYB300
GT Buffer	1.5 ml x 2	75 ml	200 ml
PR Buffer	1 ml	15 ml	40 ml
GB Buffer	2 ml	60 ml	155 ml
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer[1] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer	1 ml	30 ml	75 ml
RNase A (50 mg/ml)[2]	25 µl	550 µl	550 µl x 3
GD Columns	4	100	300
Beadbeating Tubes Type B	4	100	300
2 ml Collection Tubes	4	100	300

[1]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

[2]RNase A is shipped at room temperature and should be stored at 4ºC for extended periods.

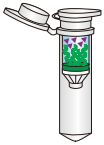
During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

Quick Protocol Diagram



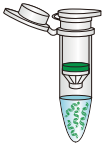
Lysis of yeast and other fungus species using beadbeating tubes and a standard vortex or bead beating instrument.

Enzymatic cell lysis using Lyticase or Zymolase is not required.



DNA binding to membrane while contaminants remain suspended

Wash (removal of contaminants while DNA remains bound to membrane)



Elution of pure genomic DNA which is ready for subsequent reactions

Presto™ Mini gDNA Yeast Kit Protocol

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

IMPORTANT BEFORE USE!

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.
2. RNase A is shipped at room temperature and should be stored at 4ºC for extended period.

Additional Requirements

absolute ethanol, 1.5 ml microcentrifuge tubes, bead beating instrument/standard vortex

Protocol Procedure

1. Sample Preparation

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.

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.

2. Lysis

Add **600 µl of GT Buffer** then re-suspend the cell pellet by vortex or pipette. Transfer the re-suspended yeast/fungus cells and 5 µl of RNase A (50 mg/ml) to a **Beadbeating Tube**. Use a bead beating instrument or attach the **Beadbeating Tubes** to a standard vortex by taping or using an adapter. Follow the instrument instruction or vortex at maximum speed for 10 minutes at room temperature. Incubate the **Beadbeating Tubes** at 70ºC for 10 minutes. During incubation, invert the tubes every 3 minutes.

Carefully open the cap then add **100 µl of PR Buffer**. Mix by vortex briefly to eliminate the foam caused by detergents. Incubate the **Beadbeating Tubes** on ice for 5 minutes. Centrifuge at 11,000 x g for 3 minutes at room temperature then transfer **450 µl of supernatant** to a clean 1.5 ml microcentrifuge tube.

NOTE: Preheat the required Elution Buffer (200 µl per sample) to 70ºC for step 5 DNA Elution.

3. DNA Binding

Add **450 µl of GB Buffer** and **450 µl of absolute ethanol** to the sample and mix IMMEDIATELY by shaking vigorously for 10 seconds.

NOTE: Equal volume of GB Buffer and absolute ethanol can be mixed in advance and stored at room temperature. Transfer 900 µl of GB Buffer and ethanol mixture to the sample then mix by shaking vigorously.

Place a **GD Column in a 2 ml Collection Tube**. Transfer **700 µl of sample mixture** to the **GD Column** then centrifuge at 16,000 x g for 1 minute at room temperature then discard the flow-through. Place the **GD Column** back in the **2 ml Collection Tube**. Transfer the remaining sample mixture to the **GD Column** and centrifuge at 16,000 x g for 1 minute at room temperature. Discard the flow-through then place the **GD Column** back in the **2 ml Collection Tube**.

4. Wash

Add **400 µl of W1 Buffer** to the **GD Column**. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the **GD Column** back in the **2 ml Collection Tube**. Add **600 µl of Wash Buffer (make sure absolute ethanol was added)** to the **GD Column**. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the **GD Column** back in the **2 ml Collection Tube**. Centrifuge at 16,000 x g for 3 minutes at room temperature to dry the column matrix.

5. Elution

Transfer the dried **GD Column** to a new 1.5 ml microcentrifuge tube. Add **100 µl of preheated Elution Buffer**[1], TE[2] or water[3] into the CENTER of the column matrix. Let stand for at least 2 minutes to allow Elution Buffer, TE or water to be completely absorbed. Centrifuge at 16,000 x g for 2 minutes at room temperature to elute the purified DNA.

[1]If a higher DNA concentration is required, use 30 µl of Elution Buffer (10 mM Tris-HCl, pH8.5) then repeat the Elution step by adding the same 30 µl of Elution Buffer (which now contains the eluted DNA) to the center of the column matrix again. If maximum DNA yield is required, use 200 µl of Elution Buffer (DNA concentration will be diluted). Ensure that Elution Buffer is added into the center of the GD Column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GD Column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the GD Column matrix and is completely absorbed.

Troubleshooting

Low Yield

Incomplete sample lysis or homogenization.

Horizontally vortex the Beadbeating Tube at maximum speed using a vortexer at room temperature for 10 minutes or use a Disruptor Genie or similar. Incubate the sample at 70ºC for 10 minutes to facilitate cell lysis following vortex.

Incorrect DNA elution.

Pre-heat the Elution Buffer to 70ºC prior to DNA elution. Make sure Elution Buffer is added to the center of the GD Column and is absorbed completely.

Incomplete buffer preparation.

Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Degraded DNA

Mechanical sample disruption is too vigorous.

Alternative lysis method for less DNA shearing: After adding Yeast/Fungus cells and GT Buffer, vortex the Beadbeating Tube at maximum speed for 5 seconds then incubate the Beadbeating Tube at 70ºC for 5 minutes. Repeat these steps 3 times. This lysis method will reduce DNA shearing but may also reduce DNA yield.

DNA Contaminated RNA

RNA carry-over.

Add 5 µl of RNase A (50 mg/ml) to the sample before cell lysis by vortex.

Eluted DNA Does Not Perform Well In Downstream Applications

Residual ethanol contamination.

Following the wash step, dry the GD Column with additional centrifugation at 16,000 x g for 5 minutes to ensure the GD Column membrane is completely dry.

Presto™ Mini gDNA Yeast Kit Test Data

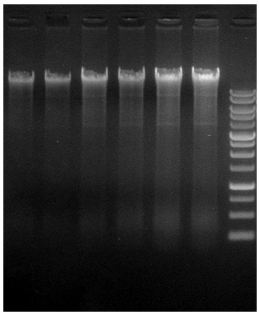


Figure 1. Genomic DNA was extracted using the Presto™ Mini gDNA Yeast Kit. *Saccharomyces cerevisiae* (50 mg, 100 mg, 200 mg) was harvested by centrifugation at 5,000 x g for 10 minutes. 5 µl aliquots of purified genomic DNA from a 100 µl eluate were analyzed by electrophoresis on a 1% agarose gel.
M = Geneaid 1 Kb DNA Ladder

Sample	ng/µl	260/280	260/230	Yield (µg)
1	21.2	1.89	2.09	2.1
2	22.2	1.90	2.05	2.2
3	37.5	1.91	2.18	3.8
4	40.7	1.90	2.05	4.1
5	75.3	1.86	2.07	7.5
6	84.9	1.87	2.11	8.5

Related DNA Extraction Products

Genomic DNA Extraction and Purification		
Product	Package Size	Catalogue Number
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	GB100/101/300/301
Genomic DNA Mini Kit (Tissue)	50/100/300 preps	GT050/100/300
gSYNC™ DNA Extraction Kit	50/100/300 preps	GS050/100/300
Genomic DNA Mini Kit (Plant)	100 preps	GP100
Genomic DNA Maxi Kit (Plant)	10/25 preps	GPM10/25
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	GBB100/300
Geneius™ Micro DNA Extraction Kit	100/300 preps	GMB100/300
Presto™ Buccal Swab gDNA Extraction Kit	100/300 preps	GSK100/300

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

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