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A Geno Technology, Inc. (USA) brand name

OmniPrep™ for Yeast

For High Quality Genomic DNA Extraction from Yeast

(Cat. #786-400)



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INTRODUCTION

The OmniPrep™ for Yeast kit isolates high quality genomic DNA from yeast. The kit isolates high purity (A_{260}/A_{280} ratios of 1.7 to 2) DNA between 100-200kbp and the yield is 3-6µg/ml yeast culture.

If used according to the protocols this kit purifies DNA from 300ml yeast culture.

ITEM(S) SUPPLIED (Cat. # 786-400)

Description	Size
Yeast Suspension Buffer	2 x 15ml
Genomic Lysis Buffer	100ml
DNA Stripping Solution	10ml
Precipitation Solution	30ml
LongLife™ RNase (5mg/ml; >60U/mg)	0.5ml
LongLife™ Zymolyase® (1.5U/µl)	2 x 0.5ml
LongLife™ Proteinase K (5mg/ml)	2 x 0.5ml
β-Mercaptoethanol	1ml
TE Buffer	20ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the kit components as recommended on the label.

ADDITIONAL REAGENT(S) REQUIRED

- Chloroform
- Isopropanol
- 70% Ethanol

PREPARATION BEFORE USE

Proteinase K Solution: Add 0.5ml sterile deionized water and briefly vortex. To avoid repeated freezing-thaw, dispense the Proteinase K solution into aliquots of 30µl/tube and freeze at -20°C.

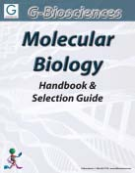
Genomic Lysis Buffer & DNA Stripping Solution: If a precipitate forms due to cold storage allow to warm to room temperature until precipitate dissolves.

PROTOCOL

1. Aliquot 1.5ml yeast overnight culture into a 1.5ml microfuge tube and centrifuge at 14,000xg for 30 seconds. Discard the supernatant.
2. Add 150µl Yeast Suspension Buffer, 5µl LongLife™ Zymolyase® and 1µl β-mercaptoethanol to the pellet and gently vortex to resuspend.
3. Incubate at 37°C for 30 minutes with periodic mixing.
4. Centrifuge for 5 minutes at 14,000xg and pour off the supernatant. Gently vortex the tube to resuspend the pellet in the residual liquid.
5. Add 500µl Genomic Lysis Buffer and mix by inverting the tube several times. Do not vortex.
6. Incubate the sample at 55-60°C for 15 minutes. Do not heat higher than 60°C
OPTIONAL: For maximum DNA recovery, add 1µl Proteinase K solution for every 100µl Lysis Buffer and incubate at 60°C for 1-2 hours. Invert the tube periodically each hour. This step will digest hard to handle tissues and significantly improve the yield.
7. Allow the sample to cool to room temperature. Add 200µl chloroform and mix by inverting the tube several times. Centrifuge for 10 minutes at 14,000xg and carefully remove the upper phase to a clean microcentrifuge tube.
8. Add 50µl DNA Stripping Solution to the sample and invert several times to mix. Incubate the sample for 5-10 minutes at 60°C.
9. Add 100µl Precipitation Solution and mix by inverting the tube several times. A white precipitate should be produced, if not add 50µl aliquots of Precipitation Solution until a white precipitate forms.
10. Centrifuge the sample at 14,000xg for 5 minutes.
11. Transfer the supernatant to a clean tube and precipitate the genomic DNA with 500µl isopropanol. Invert the tubes 10 times to precipitate the DNA.
12. Centrifuge at 14,000xg for 5 minutes to pellet genomic DNA. Remove the supernatant.
13. Add 700µl 70% ethanol to the tube and invert several times to wash the DNA pellet. Centrifuge for 1 minute at 14,000xg.
In some samples, the pellet may be hard to see at this point and will be loosely attached to the tube.
14. Decant or pipette off the ethanol wash. Invert the tube on a clean absorbent surface for several minutes to allow any excess ethanol to drain away. Do not let the pellet dry completely or it will be difficult to rehydrate.
15. Add 50-100µl TE Buffer to the pellet. Incubate at room temperature for at least 15 minutes to rehydrate. Incubating the tube at 55-60°C will speed up rehydration. Incubate for 5-60minutes.
OPTIONAL: 1µl LongLife™ RNase for every 100µl TE Buffer can be added at this stage.
16. Store DNA at 4°C, for long-term storage store at -20°C or -80°C.

RELATED PRODUCTS

Download our Molecular Biology Handbook.



<http://info.gbiosciences.com/complete-molecular-biology-handbook/>

For other related products, visit our website at www.GBiosciences.com or contact us.

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