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A Geno Technology, Inc. (USA) brand name

OmniPrep™ for Fungus

For High Quality Genomic DNA Extraction from Fungal Samples

(Cat. # 786-399)

INTRODUCTION

The OmniPrep™ for Fungus kit isolates high quality genomic DNA from fungal samples. The kit isolates high purity (A_{260}/A_{280} ratios of 1.7 to 2) DNA between 100-200kbp and the yield is 0.2-1µg/5mg fungal samples. If used according to the protocols this kit purifies DNA from 1-2gm fungal tissues.

ITEM(S) SUPPLIED (Cat. # 786-399)

Description	Size
Genomic Lysis Buffer	100ml
DNA Stripping Solution	10ml
Precipitation Solution	30ml
Longlife™ RNase (5mg/ml; >60U/mg)	0.5ml
LongLife™ Proteinase K (5mg/ml)	2 x 0.5 ml
Molecular Grinding Resin™	0.5ml resin
TE Buffer	20ml

STORAGE CONDITIONS

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

ADDITIONAL ITEMS REQUIRED

Chloroform, Isopropanol and 70% Ethanol
Molecular Grinding Resin™ (Cat. # 786-138), Molecular Grinding Resin™ with pestles and tubes (Cat. # 786-138PR)

PREPARATION BEFORE USE

Proteinase K Solution: 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
Molecular Grinding Resin: Centrifuge the Molecular Grinding Resin tube for 2 minutes at 2,500x g and remove the water. Add 0.5ml Genomic Lysis Buffer.

PROTOCOL FOR FUNGAL TISSUE

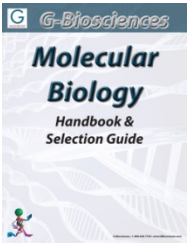
1. Collect fungal tissue from liquid culture and wash 2-3 times in sterile water.
2. Fungal mycelia are best prepared by grinding samples using Molecular Grinding Resin™ in Genomic Lysis Buffer. For fungal teliospores, grinding samples in liquid nitrogen to a fine powder and quickly add to an appropriate volume of Genomic Lysis Buffer is recommended.

NOTE: *The kit is supplied with enough Molecular Grinding Resin™ for 16 extractions as many labs have their own fungal grinding techniques. Additional Molecular Grinding Resin™ (Cat. # 786-138) and with pestles and tubes (Cat. # 786-138PR) is available.*

3. Add 10-20mg fungal mycelia to a microcentrifuge tube containing 500µl Genomic Lysis Buffer. Resuspend Molecular Grinding Resin by vigorous mixing or vortexing.
4. Add 30µl Molecular Grinding Resin™ using a wide bore pipette tips and grind with a microcentrifuge pestle. For teliospores, add ground powder to 500µl Genomic Lysis Buffer and vortex to wet sample.
5. 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.
6. 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.
7. Add 50µl DNA Stripping Solution to the sample and invert several times to mix. Incubate the sample for 5-10 minutes at 60°C.
8. 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.
9. Centrifuge the sample at 14,000xg for 5 minutes.
10. Transfer the supernatant to a clean tube and precipitate the genomic DNA with 500µl isopropanol. Invert the tubes 10 times to precipitate the DNA.
11. Centrifuge at 14,000xg for 5 minutes to pellet genomic DNA. Remove the supernatant.
12. 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.
13. 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.
14. Add 50 to 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*: Add 1µl LongLife™ RNase for every 100µl TE Buffer at this stage.*
15. 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

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Last saved: 3/15/2017 CMH

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