

ODP312

Fungal DNA Kit

For isolation of genomic DNA
from fungal cells.



Fungal DNA Kit

Spin Column
(ODP312)

Kit Contents

Contents	50 Preps
Buffer GA	10mL
Buffer GB	10mL
Buffer GD	13mL
Buffer PW	15mL
Buffer TE	10mL
Proteinase K (20mg/mL)	1mL
Spin column CB3	50
Collection Tubes (2mL)	50
Handbook	1

Storage

Fungal DNA kit could be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer term storage, the kit could be stored at 2-8°C.

(Note: Check buffers for precipitate before use and dissolve at 37°C for 10 minutes if necessary)

Proteinase K should be stored at -20°C.

Introduction

Fungal DNA Kit is based on silica membrane technology and special buffer system for many kinds of sample's genomic DNA extraction. The spin column made of new type silica membrane can bind DNA optimally on given salt and pH conditions. Simple centrifugation processing completely removes contaminants and enzyme inhibitors such as proteins and divalent cations. Extracted DNA is eluted in low-salt buffer or water, ready for use in downstream applications.

DNA extracted by Fungal DNA Kit is suited for restriction analysis, PCR analysis, Southern blotting, and cDNA library.

Important Notes

1. Add appropriate amount of ethanol (96-100%) to Buffer GD and Buffer PW as indicated on the bottle before use.

Note: It is advised to reconstitute ONLY required volume of Buffer GD with ethanol (96-100%) as reconstituted Buffer GD will precipitate on long term storage.

2. All centrifugation steps are carried out at $13,400 \times g$ (12,000rpm) in conventional tabletop microcentrifuge at room temperature.
3. Increasing the time of absorption and elution could improve recovery efficiency.
4. The recovery efficiency is related to starting DNA quantity and elution volume. The less starting quantity or elution volume, the less recovery efficiency.
5. If a precipitate has formed in Buffer GB or Buffer GD, warm buffer to 56°C until the precipitate has fully dissolved.
6. Repeated freezing and thawing of Proteinase K should be avoided; otherwise it would reduce the DNA quality and quantity.

Materials required but not supplied

1. Lyticase (10U/ μL ; Cat# ORT410). Storage at -20°C
2. Sorbitol Buffer: 1M Sorbitol in 0.1M EDTA solution (pH 7.4)

Protocol

Ensure that Buffer GD and Buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.

1. Pellet an appropriate amount of fungal cell (no more than 5×10^7 cells) in a microcentrifuge tube, centrifuge for 1 minute at 12,000rpm ($\sim 13,400 \times g$). Discard supernatant.

Note: If the sample is more than 700 μL , repeat the centrifugation process to collect all fungal cells into one tube.

2. Enzymatic Lysis: Add 600 μL Sorbitol Buffer and 50U Lyticase. Mix thoroughly, and incubate at 30°C for 30 minutes. Centrifuge for 10 minutes at 4,000rpm ($\sim 1500 \times g$). Discard supernatant.

Note: Above protocol is optimized for processing 5×10^7 fungal cells. Lysis time and Lyticase concentration should be varied according to the type of strain and amount of fungal cell processed.

3. Add 200 μL Buffer GA. Mix thoroughly by vortexing.

Note: If RNA-free genomic DNA is required, add 4 μL RNase A (10mg/mL) (ORT405-01), mix by vortexing for 15 seconds, and incubate for 5 minutes at room temperature ($15-25^{\circ}\text{C}$).

4. Add 20 μL Proteinase K, mix thoroughly by vortexing.
5. Heat the sample at 56°C for 10 minutes to yield a homogeneous solution.
6. Add 200 μL Buffer GB to the sample, mix thoroughly by vortexing, and incubate at 70°C for 10 minutes to yield a homogeneous solution. Briefly centrifuge the 1.5mL microcentrifuge tube at 1,000 - 3,000rpm for 30 seconds to remove drops from the inside of the lid.

Note: Precipitates are expected, but it will not interfere with the extraction.

7. Add 200µL ethanol (96-100%) to the sample, and mix thoroughly by vortexing for 20 seconds. Briefly centrifuge the 1.5mL microcentrifuge tube at 1,000 - 3,000rpm for 30 seconds to remove drops from the inside of the lid.

Note: Precipitate formed in the earlier step will be dissolved.

8. Pipette the mixture from step 7 into the spin column CB3 (in a 2mL collection tube) and centrifuge at 12,000rpm (~13,400 ×g) for 45 seconds. Discard flow-through and place the spin column into the collection tube.
9. Add 500µL Buffer GD to spin column CB3, and centrifuge at 12,000rpm (~13,400 ×g) for 30 seconds, then discard the flow-through and place the spin column into the collection tube.

Note: It is advised to reconstitute ONLY required volume of Buffer GD with ethanol (96-100%) as reconstituted Buffer GD will precipitate on long term storage.

10. Add 700µL Buffer PW to spin column CB3, and centrifuge at 12,000rpm (~13,400 ×g) for 30 seconds. Discard the flow-through and place the spin column into the collection tube.

Note: A second wash with 700µL PW is recommended, if the salts have not been completely removed from the spin column.

11. Centrifuge at 12,000rpm (~13,400 ×g) for 2 minutes to dry the membrane completely.

Note: The resident ethanol of Buffer PW may have some affect in downstream application.

12. Place the spin column CB3 in a new clean 1.5mL microcentrifuge tube, and pipette 50-200µL Buffer TE or distilled water directly to the center of the membrane. Incubate at room temperature (15–25°C) for 2-5 minutes, and then centrifuge for 2 minutes at 12,000rpm (~13,400 ×g).

Note: To increase the DNA yield, introduce the eluted Buffer TE to the column and centrifuge for 2 minutes at 12,000 rpm. If the volume of eluted buffer is less than 50µL, it may affect recovery efficiency. What's more, the pH value of eluted buffer will have some influence in eluting, we suggest Buffer TE or distilled water (pH 7.0 - 8.5) to elute genomic DNA. For long-term storage of DNA, eluting in Buffer TE and storing at –20°C is recommended, since DNA stored in water is subject to acid hydrolysis.