YEAST DNA PURIFICATION KIT

User Manual

26-003.....5 preps
26-009.....50 preps
26-009B.....50 preps
P/N 03-293-3 REV. F

OMNI INTERNATIONAL

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INTRODUCTION

The Omni Yeast DNA Kit allows for the rapid and reliable isolation of high-quality total cellular DNA from a wide variety of yeast species. Up to 3 mL log-phase culture (OD_{600} of 10 in YPD medium) can be processed. The system combines the reversible nucleic acid binding properties of Omni DNA Mini Column matrix with the speed and versatility of spin column technology to yield approximately 15-30 µg DNA with an A_{260}/A_{280} ratio of 1.7-1.9. Purified DNA is suitable for PCR, restriction enzyme digestion, and hybridization applications.

OVERVIEW

If using the Omni Yeast DNA Kit for the first time, please read this manual before beginning the procedure. Yeast cells are grown to log-phase and spheroblasts are subsequently prepared. Following lysis, binding conditions are adjusted and the sample is applied to an Omni DNA Mini Column. Three rapid wash steps remove trace salts and protein contaminants. DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

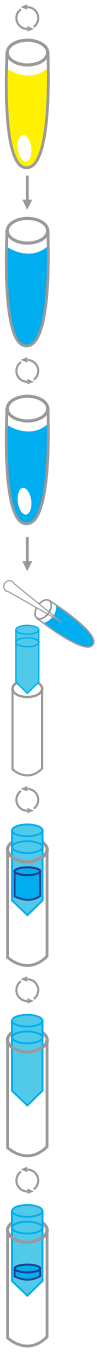
KIT COMPONENTS

Product Number	26-003	26-009	26-009B
Purification	5 Preps	50 Preps	50 Preps
Omni DNA Mini Columns	5	50	50
2 mL Collection Tubes	10	100	100
DLB Buffer	5 mL	20 mL	20 mL
BB Buffer	5 mL	20 mL	20 mL
CBH Buffer	4 mL	25 mL	25 mL
DW Buffer	1.5 mL	20 mL	20 mL
EB Buffer	2 mL	30 mL	30 mL
LD Buffer	3 mL	120 mL	120 mL
Lyticase	1,400 units	14,000 units	14,000 units
Protease Solution	150 µL	1.5 mL	1.5 mL
RNase A	30 µL	275 µL	275 µL
2 mL bead kit 0.5 mm glass	5		50
Antifoam	1 mL		1 mL
User Manual	✓	✓	✓

STORAGE AND STABILITY

Protease Solution can be stored at room temperature for 12 months. For long-term storage (>12 months), store Protease Solution at 2-8°C. Store RNase A at -20°C. Under cool ambient conditions, a precipitate may form in the DLB Buffer and/or BB Buffer. If a precipitate is present, heat the bottle at 37°C to dissolve.

ILLUSTRATED PROTOCOLS



Pellet the yeast cells and add Lyticase to remove the cell wall

Lyse and homogenize

Adjust the binding conditions

Transfer lysate into a DNA mini column to bind DNA

Wash column x 3

Dry the column at the full speed for 2 min

Elute DNA

BEFORE STARTING

Prepare Lyticase, LD buffer, DW Buffer, and CHB Buffer according to the "Preparing Reagents" section.

Heat water baths, incubators, and heat blocks to 30°C, 55°C, and 65°C

Heat EB Buffer to 65°C.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 10,000 x g
- Waterbaths, incubators, or heatblocks capable of 30°C, 55°C, and 65°C
- Nuclease-free 1.5 or 2 mL microcentrifuge tubes
- 100% ethanol
- Isopropanol
- β-mercaptoethanol (βME)
- Vortexer or Bead Mill Homogenizer

PREPARING REAGENTS

1. Prepare enough LD Buffer with β-mercaptoethanol that will be used for DNA extraction. Add 10 µL β-mercaptoethanol per 1 mL LD Buffer. The mixture is stable for 1 month at room temperature.
2. Prepare a lyticase stock solution at 5,000 units/mL in LD Buffer and aliquot. Store aliquots at -20°C.

Kit	LD Buffer to be added
26-003	220 µL
26-009 and 26-009B	2.2 mL

3. Dilute DW Buffer with 100% ethanol as follows and store at room temperature

Kit	100% Ethanol to be added
26-003	6 mL
26-009 and 26-009B	80 mL

4. Dilute CHB Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be added
26-003	1.6 mL
26-009 and 26-009B	10 mL

OMNI YEAST DNA KIT DIRECTIONS

1. Culture yeast in YPD medium to an OD_{600} of 10.
2. Centrifuge ≤3 mL culture (< 2 x 10^7) at 4,000 x g for 10 minutes.
3. Aspirate and discard the medium.
4. Resuspend cells in 480 µL LD Buffer and 40 µL lyticase solution.

Note: β-mercaptoethanol must be added to the LD Buffer before preparing the lyticase solution. Please refer to the "Preparing Reagents" section for instructions.

5. Incubate at 30°C for at least 30 minutes.
6. Centrifuge at 500 x g for 10 minutes to pellet the spheroblasts.
7. Carefully aspirate and discard supernatant. Incomplete removal of supernatant will prevent lysis of spheroblasts in the next step

26-009 - Cell Digestion

8. Resuspend pellet in 200 µL DLB Buffer. Transfer entire contents to a clean 1.5 mL microcentrifuge tube (not provided) and add 25 µL Protease Solution. Vortex 10 seconds.
9. Incubate at 55°C in a shaking water bath for 1 hr to completely lyse the cells.

26-009B - Cell Homogenization

8. Resuspend pellet in 200 µL DLB Buffer and 10 µL Antifoam reagent Transfer entire contents to a 2 mL tube containing 0.5 mm glass beads. Homogenize cells on a bead mill at 2.6 - 3.1 m/s for 30-60 seconds or with continuous vortexing for 10 minutes.
9. Add 25 µL Protease Solution. Vortex 10 seconds. Incubate at 55°C in a shaking water bath for 30 minutes to completely lyse the cells.

Note: Increased enzyme incubation times or increased mechanical dissociation time may be required to fully dissociate cells.

10. Add 5 µL RNase A. Invert tube several times to mix. Let sit a room temperature for 5 minutes.
11. Centrifuge at 10,000 x g for 5 minutes. Carefully aspirate the supernatant and transfer to a clean microcentrifuge tube.
12. Add 220 µL BB Buffer. Vortex for 15 seconds.

Note: A wispy precipitate may form upon addition of BB Buffer; it does not interfere with DNA recovery.

13. Incubate at 65°C for 10 minutes.
14. Add 220 µL 100% ethanol. Vortex for 20 seconds. If any precipitates can be seen at this point, break the precipitates by pipetting up and down 10 times.
15. Insert an Omni DNA Mini Column into a 2 mL Collection Tube.
16. Transfer the entire sample from Step 15 to the column, including any precipitates that may have formed.
17. Centrifuge at 10,000 x g for 1 minute.
18. Discard the filtrate and the Collection Tube.
19. Insert Omni DNA Mini Column into a new 2 mL Collection Tube.
20. Add 500 µL CBH Buffer.

Note: CBH Buffer must be diluted with isopropanol before use. Please see "Preparing Reagents" for instructions.

21. Centrifuge at 10,000 x g for 1 minute.
22. Discard the filtrate and reuse the Collection Tube.
23. Add 700 µL DW Buffer.

Note: DW Buffer must be diluted with 100% ethanol according to the instructions in the "Preparing Reagents" section.

24. Centrifuge at 10,000 x g for 1 minute.
25. Discard the filtrate and reuse the Collection Tube.
26. Repeat Steps 24-25 for a second DW Buffer wash step.
27. Centrifuge the empty Omni DNA Mini Column for 2 minutes at 10,000 x g to dry the column matrix.

Note: It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

28. Transfer the Omni DNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube.
29. Add 50-100 µL EB Buffer heated to 65°C.
30. Let sit at room temperature for 3 to 5 minutes.

Note: Incubating the Omni DNA Mini Column at 65°C rather than room temperature will give a modest increase in DNA yield per elution.

31. Centrifuge at 10,000 x g for 1 minute.

32. Repeat Steps 30-32 for a second elution step (optional).

Note: Any combination of the following steps can be used to help increase DNA yield.

- After adding the EB Buffer, incubate the column for 5 minutes.
- Increase the elution volume.
- Repeat the elution step with fresh EB Buffer (this may increase the yield, but decrease the concentration).
- Repeat the elution step using the elute from the first elution (this may increase yield while maintaining elution volume).

33. Store DNA at -20°C.

TROUBLE SHOOTING GUIDE

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free at: 1-800-776-4431

Problem	Cause	Suggestion
Clogged Column	Incomplete lysis	Add the correct volume of DLB Buffer and incubate at 55°C to obtain complete lysis. It may be necessary to extend incubation time by 30 minutes. Increase homogenization and speed. Increase incubation time with Protease
	Sample too large	Do not use greater than 3 mL culture at OD_{600} of 10 or 2 x 10^7 cell per spin column. For larger volumes, divide sample into multiple tubes.
	Incomplete removal of cell wall	Add more lyticase or extend the incubation time. It may be necessary to increase the incubation by 60 minutes.
Low DNA yield	Clogged column	See above
	Poor elution	Repeat elution or increase elution volume. Incubation of column at 65°C for 5 minutes after addition of EB Buffer may increase yields.
	Improper washing	DW Buffer must be diluted with 100% ethanol.
Low A_{260}/A_{280} ratio	Extended centrifugation during elution step	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation; it will not interfere with PCR or restriction digests.
	Incomplete mixing with BB Buffer	Repeat the procedure, this time making sure to immediately vortex the sample with BB Buffer.
	Insufficient incubation	Increase incubation time with DLB Buffer. Ensure that no visible cell clumps remain.

Problem	Cause	Suggestion
No DNA eluted	Poor cell lysis due to improper mixing with BB Buffer	Mix thoroughly with BB Buffer and incubate at 70°C prior to adding ethanol.
	Incomplete spheroblasting	Add more lyticase or extend the incubation time. It may be necessary to increase the incubation by 60 minutes.
	Ethanol not added to lysate/ BB Buffer mixture	Before applying sample to column, an aliquot of ethanol must be added. See protocol above.
	Ethanol was not added to DW Buffer	Dilute Wash Buffer with the indicated volume of ethanol before use

NOTES

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