BACTERIAL DNA PURIFICATION KIT

Product Manual

26-002.....5 preps
26-008.....50 preps
26-008B.....50 preps
P/N 03-293-2 REV. G

OMNI INTERNATIONAL

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INTRODUCTION

The Omni Bacterial DNA Kit allows rapid and reliable isolation of high-quality total cellular DNA from a wide variety of gram-positive and gram-negative bacterial species. Up to 1 x 10^9 bacterial cells can be processed. The system combines the reversible nucleic acid-binding properties of the Omni DNA mini column matrix with the speed and versatility of spin column technology to yield up to 15-30 µg of DNA with an A260/A280 ratio of 1.7-1.9. Purified DNA is suitable for PCR, restriction enzyme digestion, and hybridization applications.

Omni Bacterial DNA Kit will isolate all cellular DNA, including plasmid DNA.

Each Omni DNA Capture Column can bind approximately 100 µg genomic DNA. Using greater than 1 x 10^9 bacterial cells is not recommended.

OVERVIEW

If using the Omni Bacterial DNA Kit for the first time, please read this booklet to become familiar with the procedures. Bacterial cells are grown to log-phase and harvested. The bacterial cell wall is removed by lysozyme digestion followed by Protease digestion or mechanical disruption. 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 and 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.

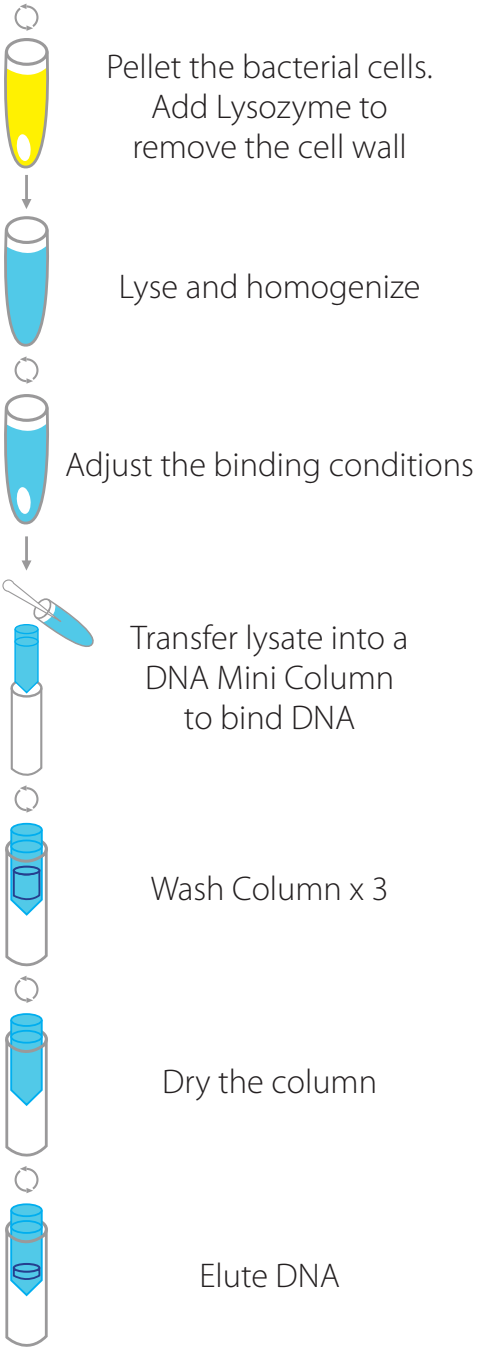
KIT CONTENTS

Product Number	26-002	26-008	26-008B
Purifications	5	50	50
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
Lysozyme	8 mg	80 mg	80 mg
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 at 2-8°C. Once reconstituted, Lysozyme must be stored at -20°C. Store RNase A at 2-8°C. Store all other components at room temperature. Check buffers for precipitates before use. Redissolve any precipitates by warming to 37˚C.

ILLUSTRATED PROTOCOLS



BEFORE STARTING

This method allows genomic bacterial isolation from up to 3 mL LB culture.

Materials and Equipment to be Supplied by User:

- Tabletop microcentrifuge
- Nuclease-free 1.5 mL microcentrifuge tubes
- Water bath capable of 37°C
- Shaking water bath capable of 55°C
- Incubator or water bath capable of 65°C
- 100% ethanol
- Isopropanol
- Tris-EDTA Buffer (TE Buffer - 10mM Tris-HCL, 0.1mM EDTA, pH 8.0)
- Vortexer or Bead Mill Homogenizer

- Prepare DW Buffer, CBH Buffer, and lysozyme as instructed in the "Preparing Reagents" section.
- Set an incubator or water bath to 65°C
- Set a water bath to 37°C
- Set a shaking water bath to 55°C
- Heat EB Buffer to 65°C

PREPARING REAGENTS

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

Kit	100% Ethanol to be added
26-002	6 mL
26-008 and 26-008B	80 mL

Dissolve Lysozyme with EB Buffer as follows and store at -20°C.

Kit	Elution Buffer to be added
26-002	100 µL
26-008 and 26-008B	1 mL

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

Kit	Isopropanol to be added
26-002	1.6 mL
26-008 and 26-008B	10 mL

OMNI BACTERIAL DNA PROTOCOL

1. Culture bacteria in LB media to log-phase. (Overnight culture can be used in many cases.)
2. Centrifuge no more than 3 mL culture or 1 x 10^9 cells at 4,000 x g for 10 minutes at room temperature.
3. Aspirate and discard the media.
4. Add 200 µL Tris-EDTA Buffer. Vortex to completely resuspend the pellet.
5. Add 10 µL Lysozyme.
6. Incubate at 37°C for 10 minutes.

Note: The amount of enzyme required and/or the length of incubation may need to be modified depending on the bacterial strain used. Complete digestion of the cell wall is essential for efficient lysis. Longer incubation time may yield better results

26-008 - Cell Digestion

7. Transfer entire contents to a clean 1.5 mL microcentrifuge tube (not provided) and add 25 µL Protease Solution and 100 µL DLB buffer. Vortex 10 seconds.
8. Incubate at 55°C in a shaking water bath for 1 hr.

26-008B - Cell Homogenization

7. Transfer entire contents to a 2 mL tube containing 0.5 mm glass beads and add 10 µL antifoam reagent and 100 µL DLB buffer. Homogenize cells on a bead mill at 2.6 - 3.1 m/s for 30-60 seconds or with continuous vortexing on highest setting for 10 minutes.
8. Add 25 µL Protease Solution. Vortex 10 seconds. Then incubate at 55°C in a shaking water bath for 30 minutes.

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

9. Add 5 µL RNase A. Invert tube several times to mix.
10. Incubate at room temperature for 5 minutes.
11. Centrifuge at 10,000 x g for 2 minutes to pellet any undigested material.
12. Transfer the supernatant to a new 1.5 mL microcentrifuge tube. Do not disturb the pellet.
13. Add 220 µL BB Buffer. Vortex to mix.
14. Incubate at 65°C for 10 minutes.

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

15. Add 220 µL 100% ethanol. Vortex for 20 seconds at maximum speed to mix thoroughly.

Note: If any precipitate can be seen at this point, break the precipitate by pipetting up and down 10 times.

16. Insert an Omni DNA Mini Column into a 2 mL Collection Tube.
17. Transfer the entire sample to the Omni DNA Mini Column, including any precipitate that may have formed.
18. Centrifuge at 10,000 x g for 1 minute.
19. Discard the filtrate and the collection tube.
20. Insert the Omni DNA Mini Column into a new 2 mL Collection Tube.
21. Add 500 µL CBH Buffer.

Note: CBH Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

22. Centrifuge at 10,000 x g for 1 minute.
23. Discard the filtrate and reuse the collection tube.
24. Add 700 µL DW Buffer.

Note: DW Buffer must be diluted with 100% ethanol before use. Please see "Preparing Reagents" for instructions.

25. Centrifuge at 10,000 x g for 1 minute.
26. Discard the filtrate and reuse the collection tube.
27. Repeat Steps 24-26 for a second DW Buffer wash step.

28. Centrifuge the empty Omni DNA Mini Column at maximum speed (≥10,000 x g) for 2 minutes to dry the column.

Note: This step is critical for removal of trace ethanol that may interfere with downstream applications.

29. Insert the Omni Mini Column into a new nuclease-free 1.5 mL microcentrifuge tube.

30. Add 50-100 µL EB Buffer heated to 65°C to the Omni DNA Mini Column.

Note: Make sure to add the EB Buffer to the center of the Omni matrix. Each 50-100 µL elution typically yields 60-70% of the DNA bound to the Omni matrix. Two elutions generally yield ~90%. However, increasing elution volume reduces the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using 50 µL EB Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 µL greatly reduce yields.

31. Let sit for 3 to 5 minutes at room temperature.

Note: Yields may be increased by incubating the column at 65°C (rather than at room temperature).

32. Centrifuge at 10,000 x g for 1 minute to elute the DNA.

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

34. Store eluted 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 to 1 hour.
	Too much sample	Do not use greater than 3 mL culture at OD600 10 or 1 x 10^9 cell per spin column. For larger volumes, divide sample into multiple tubes.
	Incomplete removal of cell wall	Add more Lysozyme or extend the incubation time. It may be necessary to increase incubation by 15 minutes.
Low DNA yield	Clogged column	See above.
	Poor elution	Repeat elution or increase elution volume (see note on Page 9). Incubation of column at 65°C for 5 minutes after addition of EB Buffer may increase yield.
	Improper washing	DW Buffer must be diluted with 100% ethanol.
	Column needs priming	Add 100 µL 3M NaOH to the column prior to loading the sample. Centrifuge at 10,000 x g for 30 seconds. Add 100 µL water to the columns and centrifuge at 10,000 x g for 30 seconds. Discard the filtrate.

NOTES

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