

# Wizard® Plus Midpreps DNA Purification System

INSTRUCTIONS FOR USE OF PRODUCTS A7640, A7651 AND A7701.

Quick  
PROTOCOL

## Midpreps Procedure (10–100ml culture volumes)

### Preparation of Cleared Lysate

1. Pellet cells at  $10,000 \times g$  for 10 minutes at  $4^{\circ}\text{C}$ .
2. Suspend pellet in 3ml Cell Resuspension Solution.
3. Add 3ml Cell Lysis Solution. Invert to mix.
4. Add 3ml Neutralization Solution. Invert to mix.\*
5. Centrifuge at  $14,000 \times g$  for 15 minutes at  $4^{\circ}\text{C}$ . Decant supernatant containing DNA.

### Plasmid DNA Purification

6. Resuspend resin. Add 10ml resin to DNA from Step 5. Swirl to mix.
7. Attach Midicolumn to vacuum manifold. Transfer resin/DNA mixture to Midicolumn. Apply vacuum, releasing when all liquid has passed through the column.\*

#### Washing

8. Add 15ml Column Wash Solution containing ethanol. Apply vacuum, pulling liquid through column. Release vacuum.
9. Repeat Step 8. After second wash, continue vacuum for 30 seconds after liquid has passed through Midicolumn. **Do not dry** longer than 30 seconds.
10. Separate Midicolumn and Reservoir. Place Midicolumn in a 1.5ml microcentrifuge tube. Centrifuge at  $10,000 \times g$  for 2 minutes.

#### Elution

11. Place Midicolumn in a new 1.5ml tube. Add 300 $\mu\text{l}$  preheated water. Wait 1 minute, then centrifuge at  $10,000 \times g$  for 20 seconds to elute DNA.  
For plasmids  $\geq 10\text{kb}$  use water preheated to  $70^{\circ}\text{C}$ ; for plasmids  $\geq 20\text{kb}$  use water preheated to  $80^{\circ}\text{C}$ .
12. Centrifuge eluate an additional 5 minutes at  $10,000 \times g$  to pellet resin fines.
13. Transfer supernatant containing DNA to a new centrifuge tube. Store at  $-20^{\circ}\text{C}$  or below.

\*For EndA+ strains and other modifications, additional protocol information in Technical Bulletin #TB173, available online at [www.promega.com](http://www.promega.com)

Overnight Culture



Centrifuge.



Resuspend pellet.  
Lyse.  
Neutralize.



Centrifuge. Save supernatant.



Mix resin, add to DNA,  
transfer to Midicolumn.

Apply vacuum.

Add wash solution. Apply vacuum. Repeat.

Separate Midicolumn from Reservoir.



Transfer Midicolumn to a 1.5ml tube.



Centrifuge.



Transfer Midicolumn to new tube. Add water.



Centrifuge.  
Transfer DNA to new tube.

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### ORDERING/TECHNICAL INFORMATION:

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