PureYield™ Plasmid Midiprep System

INSTRUCTIONS FOR USE OF PRODUCTS A2490, A2492 A2495 AND A2496.

Quick PROTOCOL

Preparation of Solutions

Before lysing cells and purifying DNA, prepare Endotoxin Removal Wash by adding isopropanol, and Column Wash by adding ethanol. Cap tightly after additions. See Technical Manual #TM253 for volumes and detailed instructions.

Standard DNA Purification Protocol

	Bacterial Culture Volume	
Prepare Lysate	**50–100ml**	**101–250ml**
1. Pellet cells at 5,000 × *g*.	10 minutes	10 minutes
2. Suspend pellet in Cell Resuspension Solution.	3ml	6ml
3. Add Cell Lysis Solution. Invert 3–5 times to mix. Incubate 3 minutes at room temperature.	3ml	6ml
4. Add Neutralization Solution. Invert 5–10 times to mix.	5ml	10ml
5. Centrifuge lysate at 15,000 × *g* at room temperature.	15 minutes	15 minutes

DNA Purification

6. Assemble a column stack by placing a blue PureYield™ Clearing Column on top of a white PureYield™ Binding Column. Place the column stack onto a vacuum manifold.
7. Carefully pour supernatant into column stack. Apply vacuum, continuing until all liquid has passed through both the clearing and binding columns.
8. Slowly release the vacuum from the filtration device. Remove the blue clearing column, leaving the binding column on the manifold.
   Note: If the binding membrane has been dislodged from the bottom of the column, tap it back into place using a sterile pipette tip.

Wash

9. Add 5.0ml of Endotoxin Removal Wash to the binding column, and allow the vacuum to pull the solution through the binding column.
10. Add 20ml of Column Wash Solution to the binding column, and allow the vacuum to pull the solution through the binding column.
11. Dry the membrane by applying a vacuum for 30–60 seconds. Repeat if the tops of the DNA binding membranes appear wet or there is detectable ethanol odor.
12. Remove the binding column from the vacuum manifold, and tap it on a paper towel to remove excess ethanol.

(Protocol continued, other side)

ORDERING/TECHNICAL INFORMATION:
www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601

Promega

Printed in USA. Revised 12/09
Part# 9FB077

PureYield™ Plasmid Midiprep System

INSTRUCTIONS FOR USE OF PRODUCTS A2490, A2492 A2495 AND A2496.

Quick PROTOCOL

DNA Purification (continued)

Elute by Vacuum (alternatively, see Elute by Centrifugation, starting at Step 18)

13. Place a 1.5ml microcentrifuge tube into the base of the Eluator™ Vacuum Elution Device (Cat.# A1071), securing the tube cap as shown in Figure 1, Panel A.
14. Assemble the Eluator™ Vacuum Elution Device, and insert the DNA binding column into the device, making sure that the column is fully seated on the collar.
15. Place the elution device assembly, including the binding column, onto a vacuum manifold (Figure 1, Panel B).
16. Add 400–600µl of Nuclease-Free Water to the DNA binding membrane in the binding column. Wait for 1 minute. Apply maximum vacuum for 1 minute or until all liquid has passed through the column.
17. Remove the microcentrifuge tube and save for DNA quantitation and gel analysis.

Elute by Centrifugation

18. Place the binding column into a new 50ml disposable plastic tube.
19. Add 600µl of Nuclease-Free Water to the DNA binding membrane in the binding column. Wait for 1 minute. Centrifuge the binding column at 1,500–2,000 × *g* for 5 minutes using a **swinging bucket rotor**, and collect the filtrate.
    Note: Do not cap the 50ml tube during centrifugation.

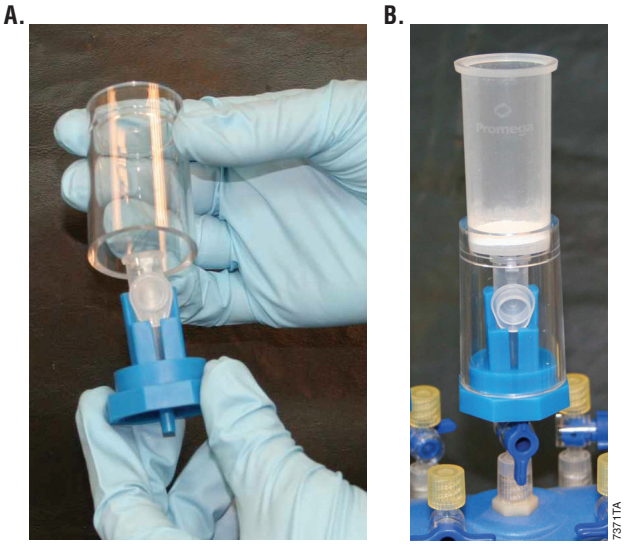

Figure 1. The Eluator™ Vacuum Elution Device for elution by vacuum. Panel A. A 1.5ml microcentrifuge tube is placed into the base of the Eluator™ Device, and the tube cap is secured in an open position, as shown. **Panel B.** The Eluator™ Vacuum Elution Device assembly, including the binding column, on a vacuum manifold.

For complete protocol information see Technical Manual #TM253, available at: **www.promega.com/tbs**

ORDERING/TECHNICAL INFORMATION:
www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601

Printed in USA. Revised 12/09.
Part #9FB077