

PureYield™ Plasmid Maxiprep System

INSTRUCTIONS FOR USE OF PRODUCTS A2391, A2392 AND A2393.

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 #TM280 for volumes and detailed instructions.

Preparation and Lysis of Bacterial Cell Cultures

Note: Perform all lysis steps at room temperature.

1. Grow 100–250ml of transformed *E. coli* cells overnight (16–21 hours).
2. Pellet cells at $5,000 \times g$ for 10 minutes. Discard the supernatant.
3. Resuspend cell pellet thoroughly in 12ml of Cell Resuspension Solution by vortexing or pipetting.
4. Add 12ml of Cell Lysis Solution. Invert gently 3–5 times to mix. Incubate for 3 minutes at room temperature.
5. Add 12ml of Neutralization Solution. Invert gently 10–15 times to mix.
6. Centrifuge the lysate at $14,000 \times g$ for 20 minutes at room temperature using a fixed angle rotor. Alternatively, centrifuge the lysate at $7,000 \times g$ for 30 minutes at room temperature.

Plasmid DNA Purification

Note: Perform all purification and elution steps at room temperature.

7. Assemble the blue PureYield™ Clearing Column and white PureYield™ Maxi Binding Column in a stack, with the clearing column on top. Place this column stack on the vacuum manifold.
8. Pour one half of the lysate into the blue PureYield™ Clearing Column.
9. Apply maximum vacuum until the lysate has passed through both the clearing and binding columns.
10. Add the remaining lysate and maintain vacuum until the liquid has cleared both columns.
11. Slowly release the vacuum. Then remove and discard the blue PureYield™ Clearing Column, leaving the PureYield™ Maxi Binding Column on the vacuum manifold.

Wash

12. Add 5ml of Endotoxin Removal Wash to the PureYield™ Maxi Binding Column, apply a vacuum and allow the solution to be pulled through the column.
13. Add 20ml of Column Wash to the binding column, and allow the vacuum to pull the solution through the column.
14. Dry the membrane by applying a vacuum for 5 minutes. If the top of the membrane in the binding column does not appear dry, continue the vacuum for an additional 5 minutes. If more than six samples are being dried at once, increase the initial drying time to 10 minutes as additional samples can reduce vacuum strength.
15. Remove the PureYield™ Maxi Binding Column from the vacuum manifold, and tap the tip of the column on a paper towel to remove any remaining ethanol. Place the column into a new 50ml centrifuge tube.

(Protocol continued, other side)

ORDERING/TECHNICAL INFORMATION:

www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601




Promega

PureYield™ Plasmid Maxiprep System

INSTRUCTIONS FOR USE OF PRODUCTS A2391, A2392 AND A2393.

Quick
PROTOCOL

Elute by Vacuum (or see Elute by Centrifugation, starting at Step 21)

16. Place a 1.5ml microcentrifuge tube into the base of the Eluator™ Vacuum Elution Device (Cat.# A1071), securing the tube cap in the open position, as shown in Figure 1, Panel A.
17. 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.
18. Place the elution device assembly onto a vacuum manifold (Figure 1, Panel B).
19. Add 1ml of Nuclease-Free Water to the DNA binding membrane in the binding column. Wait 1 minute. Apply maximum vacuum for 1 minute or until all liquid has passed through the column.
20. Remove the microcentrifuge tube and save for DNA quantitation and analysis.

Elute by Centrifugation

21. Place the PureYield™ Maxi Binding Column into a new 50ml tube. Add 1.5ml of Nuclease-Free Water to the binding column, then centrifuge in a swinging bucket rotor at room temperature, 2,000 × *g* for 5 minutes. Collect the filtrate from the 50ml tube and transfer to a 1.5ml tube.

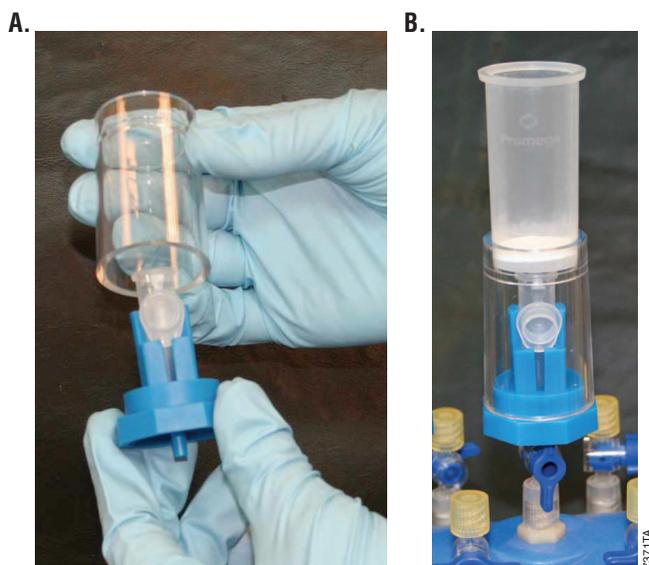


Figure 1. The Eluator™ Vacuum Elution Device for elution by vacuum. Panel A. A 1.5ml microcentrifuge tube is placed into the base of the Eluator™ Device, and the tube cap is secured in an open position, as shown. **Panel B.** The Eluator™ Vacuum Elution Device assembly, including the binding column,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

For complete protocol information see Technical Manual #TM280, available at: www.promega.com/tbs

ORDERING/TECHNICAL INFORMATION:

www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601

©2006, 2008, 2009 Promega Corporation. All Rights Reserved.
Prices and specifications subject to change without prior notice.



Promega

Printed in USA. Revised 7/09.
Part #9FB083