

Wizard® Plus Maxipreps DNA Purification System

INSTRUCTIONS FOR USE OF PRODUCTS A7270, A7401 AND A7421.

Quick
PROTOCOL

Maxipreps Procedure (100–500ml culture volumes)

Preparation of Cleared Lysate

1. Pellet cells at $5,000 \times g$ for 10 minutes at room temperature.*
2. Suspend pellet in 15ml Cell Resuspension Solution.
3. Add 15ml Cell Lysis Solution. Invert to mix.
4. Add 15ml Neutralization Solution. Invert to mix.†
5. Centrifuge lysate at $14,000 \times g$ for 15 minutes.*
6. Filter supernatant into a graduated cylinder. Measure volume, then transfer liquid to centrifuge bottle.
7. Add 0.5 volume isopropanol. Invert to mix.*
8. Centrifuge at $14,000 \times g$ for 15 minutes.*
9. Resuspend pellet in 2ml TE buffer.

Plasmid DNA Purification

10. Resuspend resin. Add 10ml resin to DNA from Step 9. Swirl to mix.
11. Attach Maxicolumn to vacuum manifold. Transfer resin/DNA mixture to Maxicolumn. Apply vacuum, releasing when all liquid has passed through the column.†

Washing

12. Add 25ml Column Wash Solution containing ethanol. Apply vacuum, pulling liquid through column. Release vacuum.
13. Add 5ml of 80% ethanol. Apply vacuum; continue for 1 minute after liquid has passed through Maxicolumn.
14. Transfer Maxicolumn to 50ml centrifuge tube. Centrifuge at $1,300 \times g$ for 5 minutes, using a swinging bucket rotor.*
15. Place Maxicolumn on manifold. Apply vacuum and continue for 5 minutes.

Elution

16. Place Maxicolumn in Reservoir tube provided. Add 1.5ml preheated water. Wait 1 minute, then centrifuge at $1,300 \times g$ for 5 minutes to elute DNA.
For plasmids ≥ 10 kb, use water preheated to 70°C ; for plasmids ≥ 20 kb, use water preheated to 80°C .
17. Filter eluted DNA. Centrifuge filtrate at $14,000 \times g$ for 1 minute.
18. Transfer supernatant containing DNA to a new centrifuge tube and store at -20°C or below.

*Rotor, solutions and centrifugation must be at room temperature.

†For EndA+ strains and other modifications, additional protocol information in Technical Bulletin #TB139, available online at www.promega.com

ORDERING/TECHNICAL INFORMATION:

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

Overnight Culture



Centrifuge.



Resuspend pellet.
Lyse.
Neutralize.
Centrifuge.
Filter supernatant,
add isopropanol.



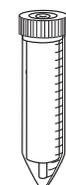
Centrifuge.



Resuspend pellet in TE buffer.
Mix with resin, transfer to
Maxicolumn. Apply vacuum.

Add wash solution, apply vacuum.

Add ethanol, apply vacuum.
Centrifuge Maxicolumn, then
dry on vacuum manifold.



Transfer Maxicolumn to Reservoir
tube. Add water, centrifuge.

Filter DNA solution.



Centrifuge.



Store DNA at -20°C or below.

2795MA11_9A



Promega

Printed in USA Revised 4/09.
Part #9FB018

© 1999, 2009 Promega Corporation. All Rights Reserved.