

Wizard® Plus Megapreps DNA Purification System

INSTRUCTIONS FOR USE OF PRODUCTS A7300, A7361 AND A7421.

Quick
PROTOCOL

Megapreps Procedure (500–1,000ml culture volumes)

Preparation of Cleared Lysate

1. Pellet cells at $1,500 \times g$ for 20 minutes at room temperature.*
2. Suspend pellet in 30ml Cell Resuspension Solution.
3. Add 30ml Cell Lysis Solution. Invert to mix.
4. Add 30ml 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 4ml TE buffer.

Plasmid DNA Purification

10. Resuspend resin. Add 20ml resin to DNA from Step 9. Swirl to mix.
11. Attach Megacolumn to vacuum manifold. Transfer resin/DNA mixture to Megacolumn. Apply vacuum and release when all liquid has passed through Megacolumn.†

Washing

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

Elution

16. Place Megacolumn in Reservoir tube provided. Add 3.0ml 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.

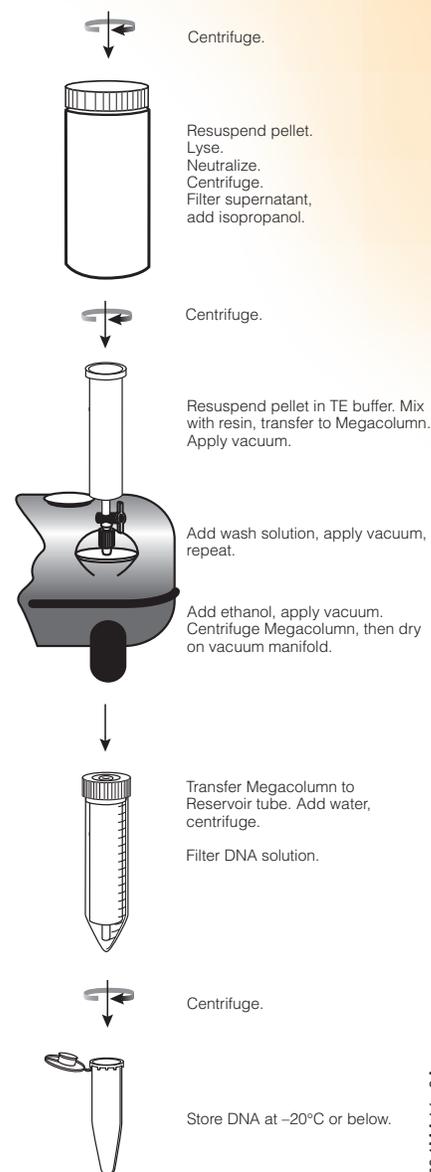
*Rotor, solutions and centrifugation must be at room temperature.

†For EndA+ strains and other modifications, additional protocol information in Technical Bulletin #TB140, available online at www.promega.com

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Overnight Culture



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