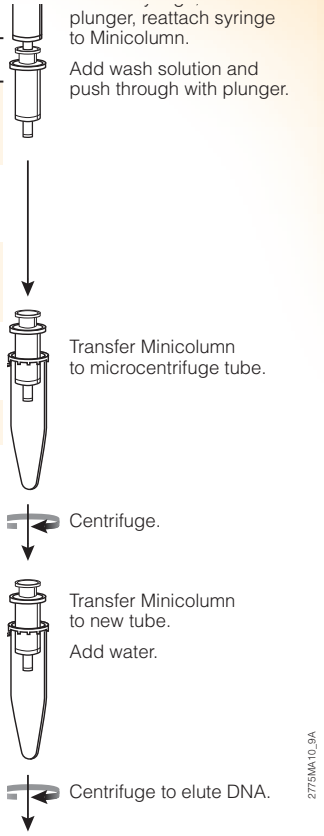
Wizard® *Plus* Minipreps DNA Purification System

INSTRUCTIONS FOR USE OF PRODUCTS A7100, A7500, A7510, A7141 AND A7211.

Quick PROTOCOL

Purification Without a Vacuum Manifold *(Using 3ml Luer-Lok® Syringes)*

	Culture Volume		
Prepare Cleared Lysate	**1–3ml**	**3–5ml**	**5–10ml**
1. Pellet cells.	1–2 minutes 10,000 × *g*†	10 minutes 10,000 × *g*†	10 minutes 1,400 × *g*
2. Suspend pellet in Cell Resuspension Solution.	200µl	300µl	400µl
3. Add Cell Lysis Solution. Invert 4 times to mix.	200µl	300µl	400µl
4. Add Neutralization Solution. Invert 4 times to mix.*	200µl	300µl	400µl
5. Centrifuge lysate for 5 minutes at 10,000 × *g*.			



Plasmid DNA Purification

6. Remove plunger from 3ml Luer-Lok® syringe (Becton-Dickinson Cat.# 9585). Attach syringe barrel to Luer-Lok® extension of Minicolumn.
7. Resuspend resin. Add 1ml resin to each Minicolumn/syringe assembly. Carefully transfer cleared lysate (from Step 5) to resin in each assembly.
8. Insert plunger and push resin and lysate into Minicolumn.*

Washing

9. Detach syringe from Minicolumn; remove plunger from syringe barrel. Reattach barrel to Minicolumn.
10. Add 2ml Column Wash Solution containing ethanol. Insert the plunger and push the Column Wash Solution through the Minicolumn.
11. Remove syringe and transfer the Minicolumn to a 1.5ml microcentrifuge tube. Centrifuge at 10,000 × *g* for 2 minutes.

Elution

12. Transfer Minicolumn to a new microcentrifuge tube.
13. Add 50µl of Nuclease-Free Water to the Minicolumn and wait 1 minute.
    For plasmids ≥10kb, use water preheated to 70°C; for plasmids ≥20kb, use water preheated to 80°C.
14. Centrifuge at 10,000 x *g* for 20 seconds at room temperature.
15. Remove and discard Minicolumn. Store DNA at –20°C or below.

†*Maximum speed on a microcentrifuge.*

*For EndA+ strains and other modifications, additional protocol information is available in Technical Bulletin #TB117, available from Promega or online at **www.promega.com**

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Plasmid DNA Purification

6. Resuspend resin. Add 1ml resin to each Minicolumn/syringe assembly. Carefully transfer cleared lysate (from Step 5) to resin in each assembly.
7. Open stopcocks. Apply vacuum to pull liquid through column. Release vacuum when all liquid has passed through column.*

Washing

8. Add 2ml Column Wash Solution containing ethanol. Apply vacuum to pull liquid through column. Continue vacuum for additional 30 seconds to dry resin.
9. Remove syringe barrel; transfer Minicolumn to a 1.5ml microcentrifuge tube. Centrifuge at 10,000 × *g* for 2 minutes.

Elution

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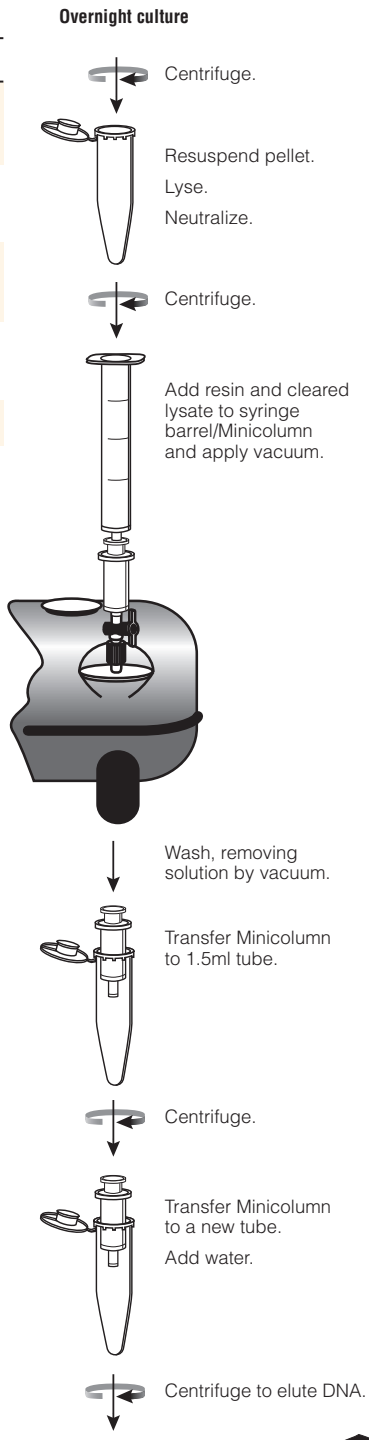

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