Wizard® PCR Preps DNA Purification System

INSTRUCTIONS FOR USE OF PRODUCTS A7170, A2180, A7181, A7241 AND A7211.

Quick PROTOCOL

Purification With a Vacuum Manifold

PCR Product Preparation

A. Extraction from Low- or High-Melting-Temperature TAE Agarose Gels

1. Following electrophoresis quickly excise the DNA band from the gel.
2. Transfer the agarose slice that is 300mg or less to a 1.5ml microcentrifuge tube, and add 1ml of resin. Incubate at 65°C for 5 minutes or until agarose is melted.

B. Extraction from Polyacrylamide Gels

1. Following electrophoresis, excise the DNA band from the gel, and place in a 1.5ml microcentrifuge tube.
2. Add 100µl of TE buffer, and incubate for ≥ 30 minutes at 37°C. Transfer 100µl of the aqueous phase to a clean tube, and add 1ml of resin. Vortex for 20 seconds.

C. Direct Purification from PCR Amplifications

1. Dispense 100µl of Direct PCR Purification Buffer into a tube. Add 30–300µl of the PCR. Vortex briefly to mix.
2. Add 1ml of resin, and vortex briefly 3 times over a 1-minute interval.

PCR Product Purification

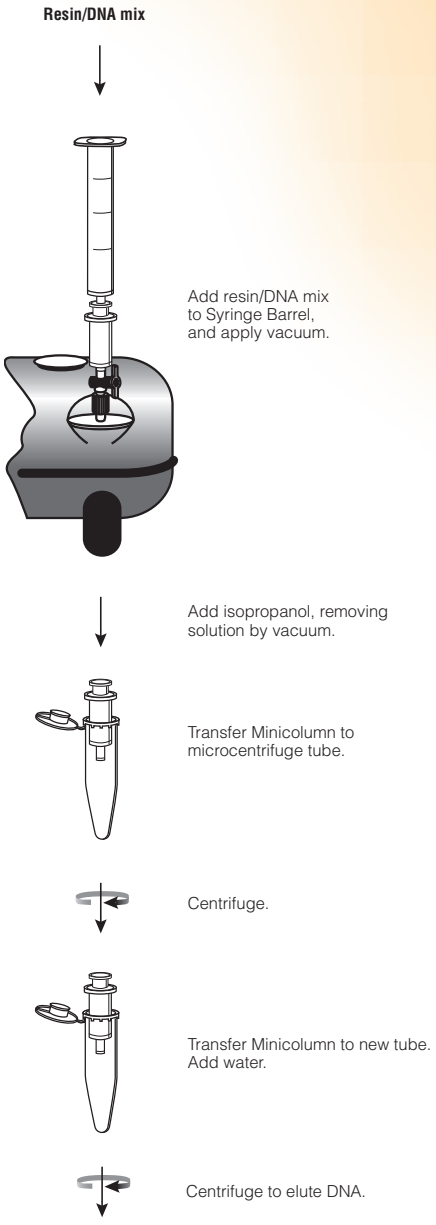
1. Prepare a Wizard® Minicolumn for each sample. Attach the Syringe Barrel to the Minicolumn. Insert the Minicolumn/Syringe Barrel into the Vacuum Manifold.
2. Add the resin/DNA mix to the Syringe Barrel.
3. Apply vacuum to pull liquid through the Minicolumn. Release vacuum when all the liquid has passed through the Minicolumn.

Washing

4. Add 2ml of 80% isopropanol to the Syringe Barrel. Apply vacuum to pull solution through the Minicolumn.
5. Dry the resin by continuing to apply the vacuum for 30 seconds.
6. Remove the Syringe Barrel, and transfer the Minicolumn to a 1.5ml microcentrifuge tube.
7. Centrifuge at 10,000 × *g* for 2 minutes.

Elution

8. Transfer the Minicolumn to a clean 1.5ml microcentrifuge tube.
9. Add 50µl of Nuclease-Free Water or TE buffer, and wait 1 minute.
   For fragments >3kb, use Nuclease-Free Water preheated to 65°C.
10. Centrifuge at 10,000 × *g* for 20 seconds at room temperature.
11. Remove and discard Minicolumn. Store DNA at –20°C or below.



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Additional protocol information is available in Technical Bulletin #TB118, available online at:
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Wizard® PCR Preps DNA Purification System

INSTRUCTIONS FOR USE OF PRODUCTS A7170, A2180, A7181, A7241 AND A7211.

Quick PROTOCOL

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

PCR Product Preparation

A. Extraction from Low- or High-Melting-Temperature TAE Agarose Gels

1. Following electrophoresis quickly excise the DNA band from the gel.
2. Transfer the agarose slice that is 300mg or less to a 1.5ml microcentrifuge tube, and add 1ml of resin. Incubate at 65°C for 5 minutes or until agarose is melted.

B. Extraction from Polyacrylamide Gels

1. Following electrophoresis, excise the DNA band from the gel, and place in a 1.5ml microcentrifuge tube.
2. Add 100µl of TE buffer, and incubate for ≥ 30 minutes at 37°C. Transfer 100µl of the aqueous phase to a clean tube, and add 1ml of resin. Vortex for 20 seconds.

C. Direct Purification from PCR Amplifications

1. Dispense 100µl of Direct PCR Purification Buffer into a tube. Add 30–300µl of the PCR. Vortex briefly to mix.
2. Add 1ml of resin, and vortex briefly 3 times over a 1-minute interval.

PCR Product Purification

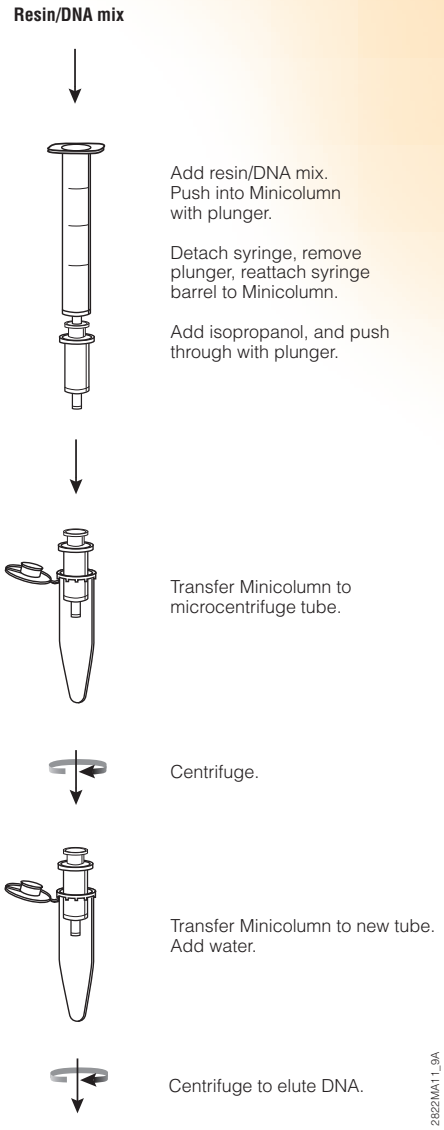
1. Prepare a Wizard® Minicolumn for each sample to be purified.
2. Remove plunger from 3ml Luer-Lok® syringes (Becton, Dickinson Cat.# 9585). Attach the Syringe Barrel to the Minicolumn.
3. Add the resin/DNA mix to the syringe barrel.
4. Insert the plunger, and push the resin/DNA slurry into the Minicolumn.*

Washing

5. Detach syringe from Minicolumn; remove plunger from Syringe Barrel. Reattach barrel to Minicolumn.
6. Add 2ml 80% isopropanol. Insert the plunger, and push the isopropanol through the Minicolumn.
7. Remove syringe, and transfer the Minicolumn to a 1.5ml microcentrifuge tube. Centrifuge at 10,000 × *g* for 2 minutes.

Elution

8. Transfer Minicolumn to a clean 1.5ml microcentrifuge tube.
9. Add 50µl of Nuclease-Free Water or TE buffer, and wait 1 minute.
   For fragments >3kb, use Nuclease-Free Water preheated to 65°C.
10. Centrifuge at 10,000 × *g* for 20 seconds at room temperature.
11. Remove and discard Minicolumn. Store DNA at –20°C or below.



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