

DNA Purification by Centrifugation

Gel Slice and PCR Product Preparation

A. Dissolving the Gel Slice

- 1. Following electrophoresis, excise DNA band from gel and place gel slice in a 1.5ml microcentrifuge tube.
- 2. Add 10µl Membrane Binding Solution per 10mg of gel slice. Vortex and incubate at 50–65°C until gel slice is completely dissolved.

B. Processing PCR Amplifications

1. Add an equal volume of Membrane Binding Solution to the PCR amplification.

Binding of DNA

- 1. Insert SV Minicolumn into Collection Tube.
- 2. Transfer dissolved gel mixture or prepared PCR product to the Minicolumn assembly. Incubate at room temperature for 1 minute.
- 3. Centrifuge at $16,000 \times g$ for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.

Washing

- 4. Add 700 μ l Membrane Wash Solution (ethanol added). Centrifuge at 16,000 \times g for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.
- 5. Repeat Step 4 with 500 μ l Membrane Wash Solution. Centrifuge at 16,000 \times g for 5 minutes.
- Empty the Collection Tube and recentrifuge the column assembly for 1 minute with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol.

Elution

- 7. Carefully transfer Minicolumn to a clean 1.5ml microcentrifuge tube.
- 8. Add 50 μ l of Nuclease-Free Water to the Minicolumn. Incubate at room temperature for 1 minute. Centrifuge at 16,000 \times g for 1 minute.
- 9. Discard Minicolumn and store DNA at 4°C or -20°C.

Additional protocol information is available in Technical Bulletin #TB308, available online at: www.promega.com



Prepare gel slice or PCR product.



Add dissolved gel mixture or prepared PCR product to SV Minicolumn assembly.



Centrifuge.



Wash, removing solution by centrifugation.



Elute DNA.

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Wizard® SV Gel and PCR Clean-Up System

INSTRUCTIONS FOR USE OF PRODUCTS A9280, A9281, A9282 AND A9285.



DNA Purification by Vacuum

Gel Slice and PCR Product Preparation

A. Dissolving the Gel Slice

- 1. Following electrophoresis, excise DNA band from gel and place gel slice in a 1.5ml microcentrifuge tube.
- 2. Add 10µl Membrane Binding Solution per 10mg of gel slice. Vortex and incubate at 50–65°C until gel slice is completely dissolved.

B. Processing PCR Amplifications

1. Add an equal volume of Membrane Binding Solution to the PCR amplification.

Binding of DNA

- 1. Attach Vacuum Adapter to manifold port and insert SV Minicolumn into Adapter.
- 2. Transfer dissolved gel mixture or prepared PCR product to the Minicolumn. Incubate at room temperature for 1 minute.
- 3. Apply vacuum to pull liquid through Minicolumn. Release vacuum when all liquid has passed through Minicolumn.

Washing

- 4. Add 700µl Membrane Wash Solution (ethanol added). Apply a vacuum to pull solution through Minicolumn.
- 5. Turn off vacuum and repeat Step 4 with 500µl Membrane Wash Solution. Apply a vacuum to pull solution through Minicolumn.
- 6. Transfer Minicolumn to a Collection Tube. Centrifuge at $16,000 \times g$ for 5 minutes.
- 7. Empty the Collection Tube and recentrifuge the column assembly for 1 minute with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol.

Elution

- 8. Carefully transfer Minicolumn to a clean 1.5ml microcentrifuge tube.
- 9. Add 50 μ l of Nuclease-Free Water to the Minicolumn. Incubate at room temperature for 1 minute. Centrifuge at 16,000 \times g for 1 minute.
- 10. Discard Minicolumn and store DNA at 4°C or -20°C.

Additional protocol information is available in Technical Bulletin #TB308, available online at www.promega.com

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Prepare gel slice or PCR product.

Attach Vacuum Adapter to manifold and insert Minicolumn. Transfer dissolved gel mixture or prepared PCR product.

Wash, removing solution by vacuum.

Transfer Minicolumn to a Collection Tube.

Centrifuge.

Elute DNA



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