

Wizard® SV Gel and PCR Clean-Up System

Instructions for Use of Products A9280, A9281, A9282 AND A9285.

Quick Protocol

DNA Purification by Centrifugation

Gel Slice and PCR Product Preparation

A. Dissolving the Gel Slice

- Following electrophoresis, excise DNA band from gel and place gel slice in a 1.5ml microcentrifuge tube.
- Add 10µl of 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

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

Binding of DNA

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

Washing

- Add 700µl of Membrane Wash Solution (ethanol added). Centrifuge at 16,000 × g for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.
- Repeat Step 4 with 500µl of Membrane Wash Solution. Centrifuge at 16,000 × 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

- Carefully transfer Minicolumn to a new 1.5ml microcentrifuge tube.
- Add 50µl of Nuclease-Free Water to the Minicolumn. Incubate at room temperature for 1 minute. Centrifuge at 16,000 × g for 1 minute.
- Discard Minicolumn and store DNA at 4°C or –20°C.



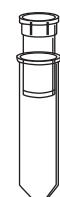
Prepare gel slice or PCR product.



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



Centrifuge.



Wash, removing solution by centrifugation.



Elute DNA.

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Quick Protocol

DNA Purification by Vacuum

Gel Slice and PCR Product Preparation

A. Dissolving the Gel Slice

- Following electrophoresis, excise DNA band from gel and place gel slice in a 1.5ml microcentrifuge tube.
- Add 10µl of 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

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

Binding of DNA

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

Washing

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

Elution

- Carefully transfer Minicolumn to a clean 1.5ml microcentrifuge tube.
- Add 50µl of Nuclease-Free Water to the Minicolumn. Incubate at room temperature for 1 minute. Centrifuge at 16,000 × g for 1 minute.
- 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

