Promega

Technical Bulletin

Wizard® SV 96 PCR Clean-Up System

INSTRUCTIONS FOR USE OF PRODUCTS A9340, A9341, A9342 AND A9345.

www.promega.com

PRINTED IN USA.
Revised 3/09

Part# TB311

Promega

Wizard® SV 96 PCR Clean-Up System

All technical literature is available on the Internet at www.promega.com/tbs
Please visit the web site to verify that you are using the most current version of this Technical Bulletin. Please contact Promega Technical Services if you have questions on use of this system. E-mail techserv@promega.com.

1. Description 1
2. Product Components 3
3. General Considerations 4
4. Wizard® SV 96 PCR Clean-Up Protocol 5
5. Troubleshooting 6
6. Related Products 8

1. Description

The Wizard® SV 96 PCR Clean-Up System[a] is designed to purify 100bp to 10kb PCR[b] products directly from an amplification reaction with up to 90% recovery (Figure 1). PCR products are commonly purified to remove excess nucleotides and primers. This membrane-based system allows recovery of the PCR fragments in as little as 20 minutes. The DNA can be used for automated fluorescent DNA sequencing, cloning, labeling, restriction enzyme digestion or DNA microarray analysis without further manipulation.

The Wizard® SV 96 PCR Clean-Up System is based on the ability of DNA to bind to silica membranes in the presence of chaotropic salts. Following PCR amplification, the reaction is added to the Membrane Binding Solution and bound to the silica membrane of the Binding Plate. PCR products are purified using 96-well vacuum filtration, eliminating the need for centrifugation. Washing the bound DNA requires no disassembly of the manifold, and filtrate waste products are delivered directly to a vacuum trap, eliminating the need to empty waste collection vessels during purification. After washing the bound PCR products, the DNA is eluted with Nuclease-Free Water (Figure 2). The Wizard® SV 96 PCR Clean-Up System requires the Vac-Man® 96 Vacuum Manifold (Figure 3) for manual PCR clean-up. Use of the Wizard® SV 96 PCR Clean-Up System on an automated liquid handling workstation requires a vacuum manifold specific to the workstation.

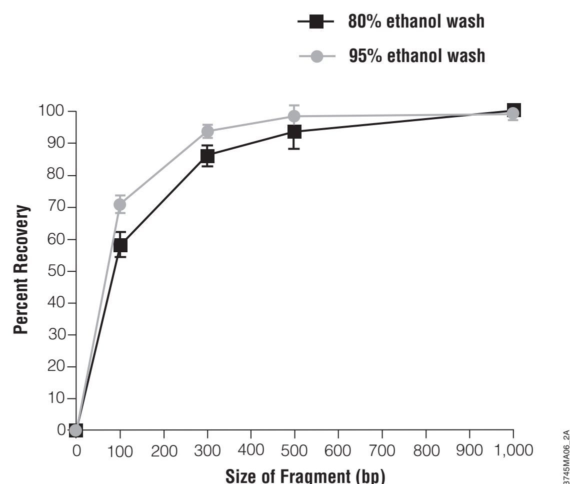


Figure 1. Percent recovery versus double-stranded DNA fragment size. For improved recovery of PCR products <500bp, use 95% ethanol wash instead of 80% ethanol. Percent recovery was calculated by resolving purified and unpurified PCR products on an agarose gel stained with ethidium bromide. Ethidium bromide staining was quantitated using a Hitachi FMBIO® scanner.

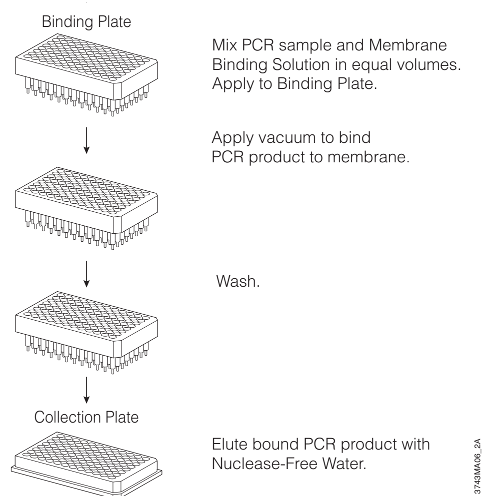
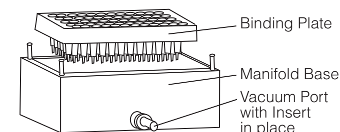
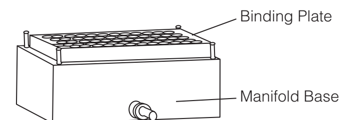


Figure 2. PCR product clean-up using the Wizard® SV 96 PCR Clean-Up System.

A. PCR Product Binding Apparatus



B. Washing Apparatus



C. Elution Apparatus

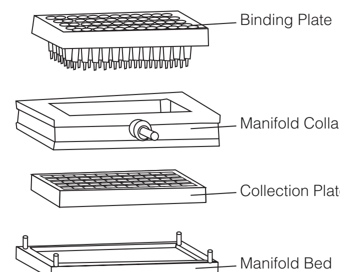


Figure 3. The Vac-Man® 96 Vacuum Manifold with the SV 96 PCR Clean-Up System. Panels A–C of this diagram show the manifold and plate combinations necessary to accomplish PCR product binding, washing and elution, respectively, for manual PCR product clean-up. For automated clean-up on the Beckman Biomek® 2000, the Beckman vacuum manifold (Beckman Part# 609670) and collar (Beckman Part# 609597) are required.

2. Product Components

Product	Size	Cat.#
Wizard® SV 96 PCR Clean-Up System	1 × 96 preps	A9340

For Laboratory Use. Each system contains sufficient reagents for 96 preps. Includes:

- 20ml Membrane Binding Solution
- 1 Binding Plate
- 25ml Nuclease-Free Water
- 1 Collection Plate

2. Product Components (continued)

Product	Size	Cat.#
Wizard® SV 96 PCR Clean-Up System	4 × 96 preps	A9341

For Laboratory Use. Each system contains sufficient reagents for 4 × 96 preps. Includes:

- 100ml Membrane Binding Solution
- 4 Binding Plates
- 150ml Nuclease-Free Water
- 4 Collection Plates

Product	Size	Cat.#
Wizard® SV 96 PCR Clean-Up System	8 × 96 preps	A9342

For Laboratory Use. Each system contains sufficient reagents for 8 × 96 preps. Includes:

- 2 × 100ml Membrane Binding Solution
- 8 Binding Plates
- 2 × 150ml Nuclease-Free Water
- 8 Collection Plates

Product	Size	Cat.#
Wizard® SV 96 PCR Clean-Up System	100 × 96 preps	A9345

For Laboratory Use. Each system contains sufficient reagents for 100 × 96 preps. Includes:

- 10 × 100ml Membrane Binding Solution
- 100 Binding Plates
- 7 × 150ml Nuclease-Free Water
- 100 Collection Plates

Storage Conditions: Store all components at room temperature (22–25°C). No refrigeration is required. Keep Membrane Binding Solution protected from light. See system label for expiration date.

3. General Considerations

The Wizard® SV 96 PCR Clean-Up System is compatible with PCR products generated using a variety of amplification enzymes, buffers or additives. Mineral oil does not interfere with purification.

4. Wizard® SV 96 PCR Clean-Up Protocol

Materials to Be Supplied by the User

- 80% ethanol (75ml/plate; freshly made before each use)
- Vac-Man® 96 Vacuum Manifold (Cat.# A2291) or suitable vacuum manifold.
- Vacuum trap for waste collection (e.g., Fisher Cat.# 10-182-50B, 1L size)
- Vacuum pump capable of 15–20 inches of Hg (e.g., Fisher Cat.# 01-092-29)
- Vacuum tubing
- Single or multichannel pipettors capable of 10–1,000µl dispensings

Comparison of Inches of Hg to Other Pressure Measurements.
15 Inches Hg
50.8kPa
381 Torr
0.501atm
7.37psi
38.1cm Hg
508mbar

Manual PCR Clean-Up Protocol

1. Prepare the vacuum manifold as shown in Figure 3. Place the Binding Plate in the vacuum manifold base. To ensure that the well numbers on the sample plate correspond to the numbers on the Binding Plate, orient the Binding Plate in the vacuum manifold with the numerical column headers toward the vacuum port. Attach the vacuum line to the vacuum port on the Manifold Base.
2. Add equal volume of Membrane Binding Solution to each PCR sample in a 96-well plate (e.g., add 100µl of Membrane Binding Solution to each 100µl PCR sample).
3. Mix by pipetting and transfer entire sample volume to the wells of the Binding Plate sitting on the vacuum manifold. Incubate for 1 minute at room temperature.
4. Apply vacuum until sample passes through the Binding Plate (approximately 30 seconds). Release vacuum.
5. Add 200µl of **freshly prepared** 80% ethanol to each well of the Binding Plate. Incubate for 1 minute at room temperature. Apply the vacuum until the ethanol passes through the plate (approximately 30 seconds). Release vacuum.

   Note: For improved recovery of PCR products <500bp, use 95% ethanol to wash instead of 80% ethanol (Figure 1).
6. Repeat Step 5 twice, for a total of 3 × 200µl ethanol washes.
7. After the wells of the Binding Plate have emptied from the final wash, continue to apply the vacuum for an additional 4 minutes to allow the binding matrix to dry.
8. Turn off vacuum. Release the vacuum line from the Manifold Base and snap it into the vacuum port in the Vacuum Manifold Collar. Remove the Binding Plate from the Manifold Base and blot by gently tapping onto a clean paper towel to remove residual ethanol.

9. Place a 96-well, U-bottom Collection Plate in the Manifold Bed and position the Vacuum Manifold Collar on top. Make sure to orient the U-bottom Collection Plate with the numerical column headers toward the vacuum port.

10. Position the Binding Plate on top of the Manifold Collar and the Collection Plate as shown in Figure 3. The Binding Plate tips must be centered on the Collection Plate wells, and both plates must be in the same orientation. Add 100µl Nuclease-Free Water to each well of the Binding Plate and incubate for 1 minute at room temperature. Apply vacuum until solution passes through the plate (approximately 1 minute).

11. Release the vacuum and remove the Binding Plate. Carefully remove the Manifold Collar, making sure that the Collection Plate remains positioned in the Manifold Bed. If droplets are present on the walls of the Collection Plate, briefly centrifuge the plate to collect the droplets on the bottom of the wells. Eluate volumes may vary but are approximately 75µl.

5. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com** E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
Low DNA yield	Incorrect ratio of Membrane Binding Solution added to the amplification reaction. Verify that an equal volume of Membrane Binding Solution was added to the reaction.
	80% ethanol not used. Verify that the ethanol solution used to wash the Binding Plate is 80% ethanol.
Poor results with automated fluorescent sequencing	Too little DNA added to the sequencing reaction. Increase the amount of DNA used in the sequencing reaction, or concentrate the DNA using ethanol precipitation. Up to 7µl of the eluted DNA can be used per fluorescent sequencing reaction.
	Too much DNA added to the sequencing reaction. Too much DNA can interfere with fluorescent sequencing. Use less eluted DNA or dilute DNA.
	TE buffer used for DNA elution. Ethanol precipitate the DNA or repurify the DNA fragments and elute with Nuclease-Free Water.

5. Troubleshooting (continued)

Symptoms	Causes and Comments
No restriction digestion or incomplete restriction digestion	Concentration of restriction enzyme or length of digestion. Increase the amount of restriction enzyme and/or length of incubation time. Digest at the appropriate temperature and in the optimal buffer for the restriction enzyme used. Keep volume of PCR to 10% or less of reaction. Use BSA at 0.2mg/ml as a protein carrier.
	Ethanol or salt carryover into the eluted DNA. Ethanol precipitate the DNA.
DNA yields on gel look low compared to spectrophotometric readings	Trace contaminants in the eluted DNA are artificially inflating the spectrophotometer readings. Use agarose gel/ethidium bromide quantitation to determine yields. Ethanol precipitate the DNA.
Low A_{260}/A_{230} ratios	Typically due to guanidine thiocyanate contamination. Low ratios do not necessarily indicate that the DNA will function poorly in downstream applications. Ethanol precipitate the DNA if the low A_{260}/A_{230} ratio is a concern.
Clogged Binding Plate	Insufficient vacuum pressure. A vacuum pressure >15 inches of Hg is required.
Purified DNA floats out of the well when loaded on a gel	Ethanol carryover. Be certain that the ethanol solution is not carried over from the wash steps into the elution step. Dry the Binding Plate for at least 4 minutes after final wash step goes through the plate.

6. Related Products

Product	Size	Cat.#
Vac-Man® 96 Vacuum Manifold	each	A2291
Membrane Binding Solution*	20ml	A9301
Wizard® SV 96 Binding Plates*	10 pack	A2271
	100 pack	A2278
PCR Master Mix*	10 reactions	M7501
	100 reactions	M7502
	1,000 reactions	M7505
Access RT-PCR System*	20 reactions	A1260
	100 reactions	A1250
	500 reactions	A1280
AccessQuick™ RT-PCR System*	20 reactions	A1701
	100 reactions	A1702
	500 reactions	A1703

*For Laboratory Use.

[a]Australian Pat. No. 730718, Singapore Pat. No. 64532, Korean Pat. No. 486402 and other patents pending.

© 2002–2009 Promega Corporation. All Rights Reserved.

Vac-Man and Wizard are registered trademarks of Promega Corporation. AccessQuick is a trademark of Promega Corporation.

Biomek is a registered trademark of Beckman Coulter, Inc. FMBIO is a registered trademark of Hitachi Software Engineering Company, Ltd.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.