TECHNICAL BULLETIN

Wizard® SV Gel and PCR Clean-Up System

Instructions for Use of Products
A9280, A9281, A9282 and A9285

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Wizard® SV Gel and PCR Clean-Up System

All technical literature is available at: www.promega.com/protocols/
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E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

The Wizard® SV Gel and PCR Clean-Up System is designed to extract and purify DNA fragments of 100bp to 10kb from standard or low-melt agarose gels in either Tris acetate (TAE) or Tris borate (TBE), or to purify PCR products directly from a PCR amplification. Up to 95% recovery is achieved depending upon the DNA fragment size (Table 1). PCR products are commonly purified to remove excess nucleotides and primers. This membrane-based system, which can bind up to 40µg DNA, allows recovery of isolated DNA fragments or PCR products in as little as 20 minutes, depending on the number of samples processed and the protocol used. The purified DNA can be used for automated fluorescent DNA sequencing, cloning, labeling, restriction enzyme digestion or in vitro transcription/translation without further manipulation.

The Wizard® SV Gel and PCR Clean-Up System is based on the ability of DNA to bind to silica membranes in the presence of chaotropic salts. After electrophoresis to separate the DNA fragments, the band(s) of interest is excised and dissolved in the presence of guanidine isothiocyanate (Membrane Binding Solution). Alternatively, after amplification, an aliquot of the PCR is added to the Membrane Binding Solution and directly purified. The system allows a choice of methods for isolation of DNA from the dissolved agarose gel slice or PCR amplification. DNA can be isolated using microcentrifugation to force the dissolved gel slice or PCR product through the membrane while simultaneously binding the DNA on the surface of the silica (Section 5.A). After washing the isolated DNA fragment or PCR product, the DNA is eluted in water. Another option is pulling the dissolved gel or PCR product through the SV Minicolumn and washing the DNA fragment using vacuum pressure (Section 5.B). The Vacuum Adapters allow the use of a vacuum manifold (e.g., Vac-Man® Laboratory Vacuum Manifold, 20-sample capacity [Cat.# A7231], or Vac-Man® Jr. Laboratory Vacuum Manifold, 2-sample capacity [Cat.# A7660]). The Vacuum Adapters (Cat. # A1331) are only supplied with Cat.# A9280, Wizard® SV Gel and PCR Clean-Up System, 10 preps, but may be purchased separately.

The Wizard® SV Gel and PCR Clean-Up System can be used with linear DNA fragments, supercoiled plasmid DNA or single-stranded linear or circular DNA. Expected yields with single-stranded DNA are lower than for double-stranded DNA.

Table 1. Percent Recovery Versus Double-Stranded DNA Fragment Size. PCR products (55–1,000bp), linearized pGEM®-3Zf(+) plasmid (3,199bp), or Lambda HindIII fragments (9,416bp and 23,130bp) were purified in triplicate from a 1% agarose gel slice in 1X TAE buffer and quantified by ethidium bromide staining.

DNA Fragment Size	Percent Recovery
55bp	26%
70bp	39%
85bp	55%
100bp	84%
500bp	89%
1,000bp	92%
3,199bp	95%
9,416bp	95%
23,130bp	47%

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Wizard® SV Gel and PCR Clean-Up System	**10 preps**	**A9280**

Each system contains sufficient reagents for 10 purifications. Includes:

- 4ml Membrane Binding Solution
- 3ml Membrane Wash Solution (concentrated)
- 1.25ml Nuclease-Free Water
- 10 Wizard® SV Minicolumns
- 10 Collection Tubes (2ml)
- 5 Vacuum Adapters

PRODUCT	SIZE	CAT.#
Wizard® SV Gel and PCR Clean-Up System	**50 preps**	**A9281**

Each system contains sufficient reagents for 50 purifications. Includes:

- 20ml Membrane Binding Solution
- 15ml Membrane Wash Solution (concentrated)
- 3.75ml Nuclease-Free Water
- 50 Wizard® SV Minicolumns
- 50 Collection Tubes (2ml)

PRODUCT	SIZE	CAT.#
Wizard® SV Gel and PCR Clean-Up System	**250 preps**	**A9282**

Each system contains sufficient reagents for 250 purifications. Includes:

- 100ml Membrane Binding Solution
- 75ml Membrane Wash Solution (concentrated)
- 13ml Nuclease-Free Water
- 250 Wizard® SV Minicolumns
- 250 Collection Tubes (2ml)

PRODUCT	SIZE	CAT.#
Wizard® SV Gel and PCR Clean-Up System	**1,000 preps**	**A9285**

Each system contains sufficient reagents for 4 × 250 purifications. Includes:

- 4 × 100ml Membrane Binding Solution
- 4 × 75ml Membrane Wash Solution (concentrated)
- 4 × 13ml Nuclease-Free Water
- 4 × 250 Wizard® SV Minicolumns
- 4 × 250 Collection Tubes (2ml)

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

3. General Considerations

Agarose, a linear polymer extracted from seaweed, is commonly used for electrophoretic separation of nucleic acids. Standard agarose melts at 87–89°C and solidifies at 36–39°C. In low-melt agarose, hydroxyethyl groups have been introduced into the polysaccharide chain, resulting in an agarose that both melts and solidifies at much lower temperatures (65°C and 24–28°C, respectively). Low-melt agarose is often used for applications that require recovery of intact DNA fragments from the gel after electrophoresis. The Wizard® SV Gel and PCR Clean-Up System can be used to recover DNA from either standard or low-melt agarose gels with no changes to the protocol or differences in recovery (Section 5).

Standard safety apparel should be worn, especially when handling ethidium bromide-stained agarose gels. This includes gloves and a UV-blocking face shield to protect the eyes and face from UV light. When excising the gel band, work quickly to minimize personal exposure to UV light and to minimize nicking of the DNA (1–4).

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

4. Gel Slice and PCR Product Preparation

Materials to Be Supplied by the User
(Solution compositions are provided in Section 8.A.)

- 1.5ml microcentrifuge tubes
- ethanol (95%)
- Vacuum Adapters (Cat.# A1331; only for vacuum purification)
- agarose gel (standard or low-melt; only for gel purification)
- 1X TAE or TBE electrophoresis buffer (only for gel purification)
- 50–65°C heating block (only for gel purification)

4.A. Preparing the Membrane Wash Solution

Add the indicated volume of 95% ethanol to the Membrane Wash Solution prior to beginning the procedure (Table 2). Mark the bottle label to record that this addition was made. Tightly close the bottle cap after each use to prevent evaporation.

Table 2. Volume of 95% Ethanol to Add to Membrane Wash Solution for Each System Size.

System Size	Part Number of Membrane Wash Solution	Volume of 95% Ethanol
10 preps	A929A	15ml
50 preps	A929B	75ml
250 preps	A929C	375ml
1,000 preps	4 × A929C	4 × 375ml

4.B. Dissolving the Gel Slice

1. Load and run the gel using an established protocol. DNA can be extracted from standard or low-melt agarose gels run with either TAE or TBE buffer.
2. Weigh a 1.5ml microcentrifuge tube for each DNA fragment to be isolated and record the weight.
3. Visualize and photograph the DNA using a long-wavelength UV lamp and an intercalating dye such as ethidium bromide. To reduce nicking, irradiate the gel for the absolute minimum time possible (1–4). Excise the DNA fragment of interest in a minimal volume of agarose using a clean scalpel or razor blade. Transfer the gel slice to the weighed microcentrifuge tube and record the weight. Subtract the weight of the empty tube from the total weight to obtain the weight of the gel slice (Notes 1–3 below).

   Note: The gel slice may be stored at 4°C or at –20°C for up to one week in a tightly closed tube under nuclease-free conditions before purification.
4. Add Membrane Binding Solution at a ratio of 10µl of solution per 10mg of agarose gel slice.
5. Vortex the mixture (Note 4) and incubate at 50–65°C for 10 minutes or until the gel slice is completely dissolved. Vortex the tube every few minutes to increase the rate of agarose gel melting. Centrifuge the tube briefly at room temperature to ensure the contents are at the bottom of the tube. Once the agarose gel is melted, the gel will not resolidify at room temperature.
6. To purify the DNA using a microcentrifuge, proceed to Section 5.A. To purify the DNA using a vacuum manifold, proceed to Section 5.B.

Notes:

1. Recovery from 1% high-melting-point agarose is comparable to that from 1–2% low-melting-point agarose. High-melting-point agarose concentrations of up to 3% have been tested. Gel slices with higher agarose concentrations (2–3%) may require a longer time to melt completely than a 1% agarose gel slice and may show reduced yields.
2. The maximum capacity of the column is 350mg of gel mass dissolved in 350µl of Membrane Binding Solution per column pass. For gel slices >350mg, continue to pass additional sample through the SV Minicolumn until all of the sample has been processed. The maximal amount of agarose that can be processed through a single column is approximately 3.5g (10 × 350mg) total.
3. The maximum binding capacity of the column is approximately 40µg per column, and as little as 10ng has been successfully purified.
4. DNA fragments that are larger than 5kb should be mixed gently to prevent shearing. Do not vortex if DNA fragment is larger than 5kb; mix by inversion.

4.C. Processing PCR Amplification Products

1. Amplify target of choice using standard amplification conditions.
2. Add an equal volume of Membrane Binding Solution to the PCR amplification (Notes 1–4 below).
3. To purify the DNA using a microcentrifuge, proceed to Section 5.A. To purify the DNA using a vacuum manifold, proceed to Section 5.B.

Notes:

1. The maximal capacity of a single SV Minicolumn is approximately 1ml of PCR amplification added to 1ml of Membrane Binding Solution (2ml total). For PCR volumes >350µl, continue to pass the sample through the column until all of the sample has been processed.
2. The maximum binding capacity is approximately 40µg per column, and as little as 10ng has been successfully purified.
3. Mineral oil does not interfere with purification.
4. For amplification reactions that do not produce a single product or where amplification has been inefficient and there is highly visible primer dimer, gel purification of the band of interest is recommended. Alternatively, an 80% ethanol wash solution can be substituted for the supplied Membrane Wash Solution to reduce primer-dimer carryover.

5. DNA Purification

Prepare the gel slice or PCR product as described in Section 4. Use either the centrifugation procedure (Section 5.A) or the vacuum procedure (Section 5.B) to recover the DNA from the dissolved gel slice or PCR amplification. After the procedure is completed, the DNA may be used in downstream applications.

5.A. DNA Purification by Centrifugation

1. Place one SV Minicolumn in a Collection Tube for each dissolved gel slice or PCR amplification.
2. Transfer the dissolved gel mixture or prepared PCR product to the SV Minicolumn assembly and incubate for 1 minute at room temperature.
3. Centrifuge the SV Minicolumn assembly in a microcentrifuge at 16,000 × *g* (14,000rpm) for 1 minute. Remove the SV Minicolumn from the Spin Column assembly, and discard the liquid in the Collection Tube. Return the SV Minicolumn to the Collection Tube.

Note: Failure to spin at 16,000 × *g* (14,000rpm) can result in reduced yield.

4. Wash the column by adding 700µl of Membrane Wash Solution, previously diluted with 95% ethanol (Section 4.A), to the SV Minicolumn. Centrifuge the SV Minicolumn assembly for 1 minute at 16,000 × *g* (14,000rpm). Empty the Collection Tube as before, and place the SV Minicolumn back in the Collection Tube. Repeat the wash with 500µl of Membrane Wash Solution, and centrifuge the SV Minicolumn assembly for 5 minutes at 16,000 × *g*.

5. Remove the SV Minicolumn assembly from the centrifuge, being careful not to wet the bottom of the column with the flowthrough. Empty the Collection Tube, and recentrifuge the column assembly for 1 minute to evaporate any residual ethanol.

6. Carefully transfer the SV Minicolumn to a clean 1.5ml microcentrifuge tube. Apply 50µl of Nuclease-Free Water directly to the center of the column without touching the membrane with the pipette tip. Incubate at room temperature for 1 minute. Centrifuge for 1 minute at 16,000 × *g* (14,000rpm).

7. Discard the SV Minicolumn, and store the microcentrifuge tube containing the eluted DNA at 4°C or –20°C.

   Note: The volume of the eluted DNA will be approximately 42–47µl. If the DNA needs to be further concentrated, perform an ethanol precipitation. Alternatively, the DNA may be eluted in as little as 15µl of Nuclease-Free Water without significant reduction in yield. If using an elution volume of 15µl, verify that the membrane is completely covered with Nuclease-Free Water before centrifugation. Elution volumes less than 15µl are not recommended (Table 3).

5.B. DNA Purification by Vacuum

1. Attach one Vacuum Adapter with a Luer-Lok® fitting to one port of the manifold (e.g., Vac-Man® or Vac-Man® Jr. Laboratory Vacuum Manifold) for each dissolved gel slice or PCR amplification. Insert SV Minicolumn into the Vacuum Adapter until it fits snugly in place.

2. Transfer the dissolved gel mixture or PCR amplification to the SV Minicolumn and incubate for 1 minute at room temperature. Apply a vacuum to pull the liquid completely through the SV Minicolumn.

   Note: The minimum vacuum pressure is 15 inches of mercury. See the table below for comparison of inches of Hg to other pressure measurements.

1 Inch Hg	15 Inches Hg
3.386kPa	50.8kPa
25.4Torr	381Torr
0.0334atm	0.501atm
0.491psi	7.37psi
2.54cm Hg	38.1cm Hg
33.86mbar	508mbar

5.B. DNA Purification by Vacuum (continued)

3. Wash the column by adding 700µl Membrane Wash Solution previously diluted with 95% ethanol (Section 4.A) to the SV Minicolumn. Make sure any droplets remaining on the sides of the SV Minicolumn from the last step are washed away. Apply a vacuum to pull the liquid through the SV Minicolumn. Repeat this wash a second time with 500µl of Membrane Wash Solution.
4. Turn off the vacuum source and open an unused port to vent the manifold. Remove the SV Minicolumn from the vacuum manifold and transfer to a Collection Tube. Centrifuge the SV Minicolumn assembly for 5 minutes at 16,000 × *g* (14,000rpm) to remove any remaining Membrane Wash Solution.
5. 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.
6. Carefully transfer the SV Minicolumn to a clean 1.5ml microcentrifuge tube, being careful not to wet the bottom of the SV Minicolumn with the flowthrough. Apply 50µl of Nuclease-Free Water directly to the center of the column without contacting the membrane. Incubate at room temperature for 1 minute. Centrifuge for 1 minute at 16,000 × *g* (14,000rpm).
7. Discard the SV Minicolumn, and store the microcentrifuge tube containing the eluted DNA at 4°C or –20°C.

   Note: The volume of the eluted DNA will be approximately 42–47µl. If the DNA needs to be further concentrated, perform an ethanol precipitation. Alternatively, the DNA may be eluted in as little as 15µl of Nuclease-Free Water without a significant reduction in yield. If using an elution volume of 15µl, verify that the membrane is completely covered with Nuclease-Free Water before centrifugation. Elution volumes less than 15µl are not recommended (Table 3).

Table 3. Percent Recovery Versus Elution Volume. A 700bp PCR product was directly purified in triplicate and quantified by ethidium bromide staining.

Elution Volume	Percent Recovery Compared to 50µl
10µl	35%
15µl	98%
25µl	98%
50µl	100%
75µl	100%
100µl	100%

6. 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	Verify that an equal volume of Membrane Binding Solution was added to the gel slice or PCR (10µl per 10mg gel slice or 10µl PCR).
	Make certain that the gel slice is completely melted before proceeding with the purification. Incubation at 50–65°C is necessary to completely melt the gel slice.
	If the amount of DNA purified is too small to quantitate by spectrophotometry, quantitate by agarose gel electrophoresis followed by ethidium bromide or PicoGreen® staining.
	Be sure to centrifuge at 16,000 × *g* (14,000rpm).
	Verify that ethanol was added to the Membrane Wash Solution (see Section 4.A), and repeat the purification.
Poor results with automated fluorescent sequencing	Too little DNA may have been used. Increase the amount of DNA used in sequencing reactions, or concentrate the DNA by ethanol precipitation. Up to 7µl of the eluted DNA can be used per fluorescent sequencing reaction.
	Too much DNA can interfere with fluorescent sequencing. Use less eluted DNA or dilute DNA prior to sequencing.
	If TE was used for elution, ethanol precipitate the DNA or repurify the DNA fragments and elute with Nuclease-Free Water.
	Excessive thymidine-dimer formation may have occurred during UV exposure. See references 1–4 for a method to minimize thymidine-dimer formation of AT-rich templates.
Poor restriction digestion	Increase the amount of restriction enzyme and/or the length of incubation time. Digest at the appropriate temperature and in the optimal buffer for the restriction enzyme used.
	Ethanol or salt carryover into the eluted DNA may have occurred. Ethanol precipitate the DNA or keep the DNA volume to 10% or less of the final reaction volume.

6. Troubleshooting (continued)

Symptoms	Causes and Comments
DNA yields on gel look low compared to spectrophotometric readings	Trace contaminants in eluted DNA can artificially inflate spectrophotometer readings. Use agarose gel electrophoresis followed by ethidium bromide or PicoGreen® staining to determine DNA yields.
	Ethanol precipitate the DNA.
Low A_{260}/A_{230} ratios	Typically due to guanidine isothiocyanate contamination. Low ratios do not necessarily indicate that the DNA will function poorly in downstream applications. If low A_{260}/A_{230} ratio is a concern, ethanol-precipitate the DNA.
Clogged spin basket	Increase the length of the 50–65°C incubation to ensure the gel slice is completely melted.
	Verify that an equal ratio of Membrane Binding Solution to gel slice mass is used (10µl per 10mg).
	A vacuum pressure of >15 inches of mercury is required to use the SV Minicolumn in the vacuum protocol. If the vacuum is insufficient, use the spin protocol.
Purified DNA floats out of the well when loaded on a gel	Ethanol carryover. Be certain that the Membrane Wash Solution is not carried over from the wash steps. If the column has been wet, empty the Collection Tube and recentrifuge the column assembly for 1 minute. Centrifuge the SV Minicolumn for 5 minutes to remove residual Membrane Wash Solution. After washing, centrifuge the column assembly with the micro-centrifuge lid open or off (Section 5.A., Step 5; Section 5.B., Step 5) to allow evaporation of any residual ethanol.
	Add 3X loading dye to the DNA sample before loading onto the gel.
Purified DNA bands are not sharp	DNA may be sheared. Mix the agarose gel slice gently with the Membrane Binding Solution.
	Nuclease contamination may be an issue. Autoclave the gel running buffer before use.
	Store the gel slice at 4°C or –20°C for no more than 1 week under nuclease-free conditions.
Low cloning efficiency	May be due to guanidine isothiocyanate contamination. Ethanol precipitate the DNA, washing the pellet with 70% ethanol to reduce contamination.

7. References

1. Zimmermann, M., Veeck, J. and Wolf, K. (1998) Minimizing the exposure to UV light when extracting DNA from agarose gels. *BioTechniques* **25**, 586.
2. Hengen, P. (1997) Methods and reagents. Protecting vector DNA from UV light. *Trends Biochem. Sci.* **22**, 182–3.
3. Grundemann, D. and Schomig, E. (1996) Protection of DNA during preparative agarose gel electrophoresis against damage induced by ultraviolet light. *BioTechniques* **21**, 898–903.
4. Cariello, N.F. *et al.* (1988) DNA damage produced by ethidium bromide staining and exposure to ultraviolet light. *Nucl. Acids Res.* **16**, 4157.

8. Appendix

8.A. Composition of Buffers and Solutions

Membrane Wash Solution
(after ethanol addition)

10mM	potassium acetate (pH 5.0)
80%	ethanol
16.7µM	EDTA (pH 8.0)

To prepare this solution, add 95% ethanol to the supplied Membrane Wash Solution (concentrated) as described in Table 2 in Section 4.A.

Membrane Binding Solution

4.5M	guanidine isothiocyanate
0.5M	potassium acetate (pH 5.0)

1X TE buffer

10mM	Tris-HCl (pH 7.5)
1mM	EDTA (pH 8.0)

1X TBE buffer

89mM	Tris base
89mM	boric acid
2mM	EDTA (pH 8.0)

1X TAE buffer

40mM	Tris base
5mM	sodium acetate
1mM	EDTA (pH 8.0)

8.B. Related Products

Product	Size	Cat.#
Vacuum Adapters	20 each	A1331
Wizard® SV 96 PCR Clean-Up System	1 × 96 preps	A9340
	4 × 96 preps	A9341
	8 × 96 preps	A9342
	100 × 96 preps	A9345
GoTaq® Green Master Mix[1]	100 reactions	M7122
	1,000 reactions	M7123
GoTaq® Colorless Master Mix[1]	100 reactions	M7132
	1,000 reactions	M7133
GoTaq® DNA Polymerase[1]	100 units	M3001
	500 units	M3005
	2,500 units	M3008
GoTaq® HotStart Polymerase[1]	100 units*	M5001
GoTaq® HotStart Green Master Mix[1]	100 reactions*	M5122
GoTaq® HotStart Colorless Master Mix[1]	100 reactions*	M5132
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
Agarose, LE, Analytical Grade	100g	V3121
	500g	V3125
Ethidium Bromide Solution, Molecular Grade	10ml	H5041
TAE Buffer,10X	1,000ml	V4271
TBE Buffer, 10X	1,000ml	V4251

[1]Different Cat.# may apply for customers in Europe. Visit **www.promega.com/catalog/** for the amplifcation product catalog numbers appropriate for your location.

*Additional sizes available.

8.C. Summary of Change

The following change was made to the 8/19 revision of this document:

Updated Section 5.A, Step 5.