Technical Bulletin

Wizard® SV 96 Plasmid DNA Purification System

INSTRUCTIONS FOR USE OF PRODUCTS A2250, A2255 AND A2291.

PRINTED IN USA.
Revised 3/09

Part# TB272

Wizard® SV 96 Plasmid DNA Purification System

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1. Description

The Wizard® SV 96 Plasmid DNA Purification System[a] provides a simple and reliable method for the rapid isolation of plasmid DNA from as many as 96 samples. The entire miniprep procedure can be completed in 60 minutes or less. The purified plasmid can be used directly for automated fluorescent DNA sequencing as well as for other standard molecular biology techniques, including restriction enzyme digestion.

Figure 1 describes plasmid DNA isolation and purification using the Wizard® SV 96 Plasmid DNA Purification System. This system requires use of the Vac-Man® 96 Vacuum Manifold (Figure 2). Plasmid DNA is purified from bacterial lysates using a 96-well vacuum filtration step to simultaneously clear the bacterial lysate and bind plasmid DNA, eliminating the need for centrifugation. Washing of the bound plasmid DNA requires removal of only

the Lysate Clearing Plate and Manifold Collar. Filtrate waste products are delivered directly to a vacuum trap, eliminating the need for emptying of waste collection vessels during plasmid DNA recovery. DNA is collected by eluting into a 96-well plate.

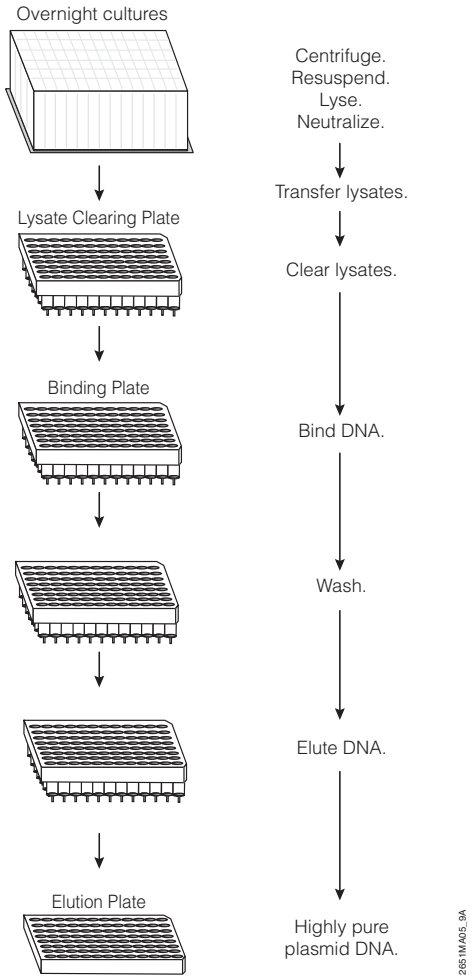


Figure 1. Flow diagram of plasmid DNA isolation and purification using the Wizard® SV 96 Plasmid DNA Purification System.

2. Product Components and Storage Conditions

Product	Size	Cat.#
Wizard® SV 96 Plasmid DNA Purification System	1 × 96 preps	A2250

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

- 40ml Wizard® SV 96 Cell Resuspension Solution
- 40ml Wizard® SV 96 Cell Lysis Solution
- 84ml Wizard® SV 96 Neutralization Solution
- 100ml Wizard® SV 96 Wash Solution
- 3ml Alkaline Protease Solution
- 13ml Nuclease-Free Water
- 1 96-Well Deep Well Plate
- 1 Wizard® SV 96 Lysate Clearing Plate
- 1 Binding Plate
- 1 Elution Plate
- 3 Plate Sealers

Product	Size	Cat.#
Wizard® SV 96 Plasmid DNA Purification System	5 × 96 preps	A2255

For Laboratory Use. Each system contains sufficient reagents for 480 isolations. Includes:

- 125ml Wizard® SV 96 Cell Resuspension Solution
- 125ml Wizard® SV 96 Cell Lysis Solution
- 425ml Wizard® SV 96 Neutralization Solution
- 370ml Wizard® SV 96 Wash Solution
- 6ml Alkaline Protease Solution
- 75ml Nuclease-Free Water
- 5 96-Well Deep Well Plates
- 5 Wizard® SV 96 Lysate Clearing Plates
- 5 Binding Plates
- 5 Elution Plates
- 15 Plate Sealers

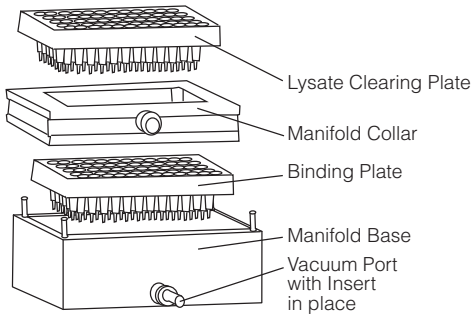
Product	Size	Cat.#
Vac-Man® 96 Vacuum Manifold	each	A2291

Storage Conditions: All Wizard® SV 96 Plasmid DNA Purification System components should be stored at 22–25°C. See the product label for expiration information.

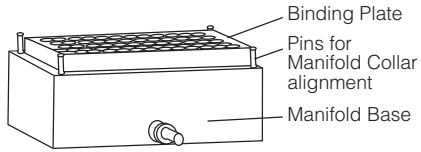
Note: Components for the Wizard® SV 96 Plasmid DNA Purification System are available for purchase individually. See Section 7 for ordering information.

Components of the Wizard® SV 96 Plasmid DNA Purification System should not be exchanged for or replaced with components from Wizard®, Wizard® *Plus* or Wizard® *Plus* SV DNA Purification Systems.

A. Lysate Clearing and DNA Binding Apparatus



B. Washing Apparatus



C. Elution Apparatus

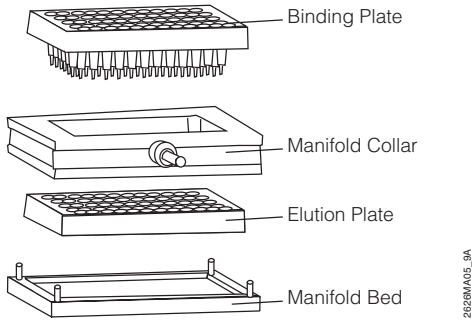


Figure 2. Diagram of the Vac-Man® 96 Vacuum Manifold with the Wizard® SV 96 Plasmid DNA Purification System components. Panels A, B and C show the manifold and plate combinations necessary to accomplish DNA binding, washing and elution, respectively.

3. Protocol for Plasmid DNA Isolation and Purification

Materials to Be Supplied by the User
(Solution compositions are provided in Section 6.)

- LB agar plates containing antibiotic
- culture medium containing antibiotic
- ethanol, 95%
- tabletop centrifuge capable of 1,500 × *g*, fitted with 96-well plate adapters (e.g., tabletop model or Beckman J2HC model# 362701 centrifuge)
- Vac-Man® 96 Vacuum Manifold
- vacuum trap for waste collection (e.g., Ansys Cat.# 584)
- vacuum pump capable of 15–20 inches of Hg (e.g., Fisher Cat.# 01-092-29)
- vacuum tubing
- **optional:** multichannel pipettors capable of dispensing 10–1,000µl

Prior to beginning the procedure with a new Wizard® SV 96 System, dilute the provided Wizard® SV 96 Wash Solution:

For the 1 × 96 system, add 170ml of 95% ethanol to the bottle of Wash Solution for a final volume of 270ml.

For the 5 × 96 system, add 630ml of 95% ethanol to the Wash Solution for a final volume of 1,000ml.

Table 1. Solution Volumes to Use Per Well with the Wizard® SV 96 DNA Purification System.

	Cell Resuspension	Cell Lysis	Neutralization	Wash	Nuclease-Free Water
Amount	250µl	250µl	350µl, then 500µl	1ml, then 1ml	100µl

3.A. Manifold Assembly

Lysate Clearing and DNA Binding (Figure 2, Panel A):

Place the Binding Plate on the Manifold Base. Connect the vacuum port in the Manifold Base to a vacuum source using the insert (provided with the manifold) and vacuum tubing. Place the Manifold Collar on top of the base and binding plate, aligning the collar with the pins. Finally, place the Lysate Clearing Plate on the Manifold Collar. **Note** that the vacuum port in the Manifold Collar is sealed because the insert has not been placed in the port.

The Lysate Clearing Plate can be identified by the **blue dot** in the top left-hand corner of the plate.

DNA Washing (Figure 2, Panel B):

Remove the Lysate Clearing Plate and Manifold Collar from the assembly in Figure 2, Panel A. The Binding Plate remains on the Manifold Base during washing.

DNA Elution (Figure 2, Panel C):

Place the Elution Plate onto the Manifold Bed, then place the Manifold Collar on the Elution Plate. Attach vacuum tubing to the insert and the insert to the vacuum port on the Manifold Collar. Place the Binding Plate on the Manifold Collar for elution of DNA into the Elution Plate.

Note: The Vac-Man® 96 Vacuum Manifold requires the use of a vacuum trap. The trap can be constructed by connecting a 500–1,000ml sidearm flask between the manifold and the vacuum pump. Alternatively, a vacuum trap assembly including rubber stopper and connector ports can be obtained commercially (e.g., Ansys Corporation, Cat.# 548; www.ansysinc.com). The use of a vacuum gauge with the vacuum assembly is also recommended.

3.B. Production of a Cleared Lysate and Binding of Plasmid DNA

1. Pellet the bacterial culture grown in a deep-well culture plate (provided) and containing high-copy-number plasmid by centrifuging for 15 minutes at 1,500 × *g* in a tabletop centrifuge. As much as 4.0 $O.D._{600}$ of total cell mass may be processed per well. Pour off the supernatant and blot the plate upside down on a paper towel to remove excess liquid. **Note:** Cell pellets may be stored at –20°C for later processing; however, storage for more than 30 days is not recommended.

2. Resuspend each cell pellet by adding 250µl of Cell Resuspension Solution. Thoroughly pipet 8–10 times until a uniform cell suspension is achieved. **Tip:** An eight- or twelve-channel pipettor may be used.

   Note: Avoid cross-contaminating samples by using a fresh pipette tip for each sample or sample set during resuspension.

3. Add 250µl of Cell Lysis Solution to each sample. Mix by tapping the plate against the palm of your hand 3–4 times. Incubate for 3 minutes at room temperature.

   Note: Allow a minimum of 3 minutes for clearing of the lysate before proceeding to Step 4. Do not incubate longer than 5 minutes.

4. **Optional:** When using an EndA+ strain of *E. coli*, add 10µl of Alkaline Protease Solution to each well and mix by tapping the side of the plate against the palm of your hand 5–10 times. Incubate for 3 minutes at room temperature. **Do not** incubate for more than 5 minutes, as nicking of the plasmid DNA may occur. See Table 2 for a list of EndA+ and EndA– strains of *E. coli*.

5. During the incubation, prepare the vacuum manifold as shown in Figure 2 and described in Section 3.A. To ensure that samples and well numbers correspond on both plates, orient the plates with the numerical column headers toward the vacuum port. Attach the vacuum line to the vacuum port on the Manifold Base.

6. Add 350µl Neutralization Solution to each sample. Mixing is not necessary. Transfer the bacterial lysates to the Lysate Clearing Plate assembled on the Vacuum Manifold (Figure 2, Panel A). Allow one minute for the filtration disks to wet uniformly, then apply a vacuum to the manifold (15–20 inches of Hg or the equivalent) using a vacuum pump fitted with a control valve. Allow 3–5 minutes under vacuum for the lysates to pass through both the Lysate Clearing Plate and the Binding Plate.

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

7. Release the vacuum. Check that the lysate has cleared both the Lysate Clearing and Binding Plates. If not, reapply the vacuum until all lysate is pulled through both plates. Remove the Clearing Plate and collar (as in washing configuration, Figure 2, Panel B).

8. Add 500µl of the Neutralization Solution to each well of the Binding Plate. Apply a vacuum for 1 minute, then turn off the pump.

3.C. Washing

9. With the Binding Plate and Manifold Base configured as in Figure 2, Panel B, add 1.0ml of Wash Solution containing ethanol to each well of the Binding Plate. Apply a vacuum for 1 minute.

10. Turn off the pump and repeat the wash procedure (Step 9). After the wells have been emptied, continue for an additional 10 minutes under vacuum to allow the binding matrix to dry.

11. Turn off the 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 tapping onto a clean paper towel to remove residual ethanol.

12. Place an Elution Plate in the Manifold Bed and position the Vacuum Manifold Collar on top. Orient the plate with the numerical column headers toward the vacuum port.

3.D. Elution

13. Position the Binding Plate on top of the Manifold Collar and Elution Plate as shown in Figure 2, Panel C. The Binding Plate tips must be centered over the Elution Plate wells and both plates must be in the same orientation. Add 100µl of Nuclease-Free Water to the **center** of each well of the Binding Plate and incubate 1 minute at room temperature. Apply a vacuum for 1 minute as previously described.

14. Release the vacuum and remove the Binding Plate. Carefully remove the Manifold Collar, making sure that the Elution Plate remains positioned in the Manifold Bed. If droplets are present on the walls of the Elution Plate

wells, centrifuge the plate briefly to collect the droplets on the bottom of the wells. Eluate volumes may vary but are generally 60–70µl. Samples can be stored at 4°C or –20°C by covering the plate tightly with a plate sealer.
Note: Upon completion of this procedure the Binding Plate can be stored at room temperature for later use of any unused wells.

Release the vacuum before removing the Vacuum Manifold Collar from the Elution Plate.

4. Supplementary Information

4.A. Selection and Preparation of Plasmids and *E. coli* Strains

Plasmid DNA can be purified from overnight cultures of *E. coli* with the Wizard® SV 96 Plasmid DNA Purification System. The yield of plasmid will vary depending on a number of factors, including the plasmid copy number, cell density of bacterial culture, type of culture medium and the bacterial strain used.

Plasmid copy number is one of the most important factors affecting plasmid DNA yield. Copy number is determined primarily by the region of DNA surrounding and including the origin of replication. This region, known as the replicon, controls replication of plasmid DNA by bacterial enzyme complexes. Some DNA sequences, when inserted into a particular plasmid, can lower the copy number of the plasmid by interfering with replication.

Dispense 1–1.2ml of culture medium containing antibiotic into the wells of the 96-well culture plate. Choose a single, well-isolated colony from a fresh agar plate containing the same antibiotic to inoculate each plate well. Cover the plate with aluminum foil or with a plate sealer that has been pierced to allow aeration of the cells. The plate should be shaken at low to moderate speed to allow aeration without causing cross-contamination. The inoculated medium should be incubated overnight (16–24 hours) at 37°C. An $O.D._{600}$ of 1.0–4.0 for high-copy-number plasmids ensures that bacteria have reached the proper growth density for harvesting and plasmid DNA isolation. Using cells at $O.D._{600}$ readings >4.0 may lead to incomplete processing of the bacterial lysate or plugging of the Clearing Plate. This may decrease yields as well as increase contaminant levels in the isolated plasmid DNA.

Note: The culture volume may vary to equal a maximum $O.D._{600}$ of 4.0 per well. It is not critical to determine the $O.D._{600}$ unless there is a possibility that the total cell mass may exceed an $O.D._{600}$ value of 4.0 per well. Cultures grown in LB medium containing antibiotics for up to 24 hours in a 96-well culture plate generally do not exceed an $O.D._{600}$ of 4.0 per well and do not need to be measured. The recommended minimum total cell mass to process per well is an $O.D._{600}$ value of 1.0.

Calculations:

$$\frac{4.0\ O.D._{600}}{O.D._{600}\text{ per 1ml}} = \text{ml of culture to obtain a total cell mass of 4.0 } O.D._{600}$$

where $O.D._{600}$ per ml = $O.D._{600}$ of culture (diluted 1:10 in medium) measured in a 1cm pathlength cuvette.

4.B. Choosing a Bacterial Strain

Endonuclease I is a 12kDa periplasmic protein that degrades double-stranded DNA. This protein is encoded by the gene *endA*. The *E. coli* genotype *endA*1 refers to a mutation in the wildtype *endA* gene, which produces an inactive form of the nuclease. *E. coli* strains with this mutation in the *endA* gene are referred to as EndA negative (EndA–). Table 2 contains a list of EndA– and EndA+ *E. coli* strains. The absence of an *endA*1 (or *endA*) in an *E. coli* genotype denotes the presence of the wildtype gene, which expresses an active endonuclease I. The wildtype is indicated as EndA+. Using Wizard® SV 96 System, high-quality DNA is easily obtained from both EndA+ and EndA– strains. However, some EndA+ strains can be problematic for a number of applications. **In general, we recommend the use of EndA– strains whenever possible, particularly for applications such as automated fluorescent sequencing.**

Table 2. EndA– and EndA+ Strains of *E. coli*.

EndA–	EndA+
BJ5183	BL21(DE3)
DH1	CJ236
DH20	HB101
DH21	JM83
DH5α™	JM101
JM103	JM110
JM105	LE392
JM106	MC1061
JM107	NM522 (all NM series strains are EndA+)
JM108	P2392

EndA–	EndA+
JM109	PR700 (all PR series strains are EndA+)
MM294	Q358
Select96™	RR1
SK1590	TB1
SK1592	TG1
SK2267	Y1088 (all Y10 series strains are EndA+)
SRB	BMH 71-18
TOP10	ES1301
XL1-Blue	
XLO	

4.C. Special Considerations for Automated Fluorescent Sequencing

For applications such as fluorescent DNA sequencing, special considerations should be given to the selection of plasmid and *E. coli* strains to optimize yield and plasmid quality. Optimal automated fluorescent sequencing results are obtained by using high-copy-number plasmids and EndA– strains of *E. coli* for plasmid propagation.

Purified plasmid DNA must be within the proper concentration range for successful automated cycle sequencing (ideally 0.2µg/µl and not less than 0.1µg/µl). Concentrations achieved with high-copy-number plasmid DNA purified using the Wizard® SV 96 System normally are of sufficient concentration for direct use in these applications; however, plasmid DNAs from low-copy-number plasmids may require concentration. The dried DNA may be suspended in 6µl of Nuclease-Free Water and the concentration again determined. When working with low-copy-number plasmids, best results are obtained by ethanol precipitation. We strongly recommend that DNA concentrations be determined by agarose gel/ethidium bromide quantitation prior to any application, particularly when using low-copy-number plasmids (1). DNA quantitation by spectrophotometric methods is prone to errors and may require a large amount of sample.

The Wizard® SV 96 System typically results in yields of 3–5µg of plasmid DNA when using a high-copy-number plasmid such as a pGEM® Vector and DH5α™ cells in 1.2ml of LB (Luria Bertani) medium containing antibiotic.

Special Considerations for Sequencing Using BigDye® Chemistry

If the BigDye® terminator ready reaction mix (Applera Corporation, Cat.# 43031-49) is diluted, it is essential to use an appropriate dilution buffer, such as 250mM Tris-HCl (pH 9.0), 10mM $MgCl_2$.

4.D. Use of Alkaline Protease

To improve the quality of plasmid DNA isolated from EndA+ strains of *E. coli*, the Wizard® SV 96 Plasmid DNA Purification System includes an alkaline protease solution. Alkaline protease, originally identified as subtilisin Carlsberg, is isolated from the bacterium *Bacillus licheniformis* (2). It is added at the end of the lysis step during the preparation of a cleared bacterial lysate to inactivate endonucleases. The alkaline protease also acts to nonspecifically degrade proteins, thus reducing the overall level of protein contaminants in the cleared bacterial lysate (3,4).

The DNA prepared by this procedure has been tested extensively in a range of molecular biology applications including fluorescent sequencing, restriction enzyme digestion and cloning.

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
Poor cell lysis	Too many bacterial cells in culture medium. All media should contain antibiotics. Process 1.0–4.0 $O.D._{600}$ cells/well.
	Poor resuspension of bacterial cell pellet. Thoroughly resuspend cell pellets prior to cell lysis. No cell clumps should be visible after resuspension.
No plasmid DNA purified	Ethanol not added to Wash Solution. Prepare Wash Solution as instructed Section 3 before beginning.
	Inaccurate quantitation of plasmid DNA yield. Quantitate plasmid DNA yield by agarose gel/ethidium bromide electrophoresis.
	DNA floats out of well during loading of gel for quantitation. Be certain to allow the full 10 minutes for drying after the final wash step to allow evaporation of any remaining ethanol. Increase loading dye concentration.
Low plasmid DNA yields	Overgrowth of bacterial culture by nontransformed cells. Do not grow the bacteria for longer than 18 hours, as antibiotic is broken down by the culture and selection is lost.
	Bacterial culture too old. Inoculate antibiotic-containing media with freshly isolated bacterial colony from an overnight plate. Incubate at 37°C for 16–18 hours.
	Low-copy-number plasmid used. Know the copy number of plasmid used; we recommend use of high-copy-number plasmids.
	Plasmid DNA yield was not accurately quantitated. Use agarose gel/ethidium bromide quantitation.
	Wrong reagents used. Make certain that Wash Solution is diluted with ethanol before use. **Note:** Wizard® and Wizard® *Plus* components should not be used with this **Wizard® SV 96** System.

5. Troubleshooting (continued)

Symptoms	Causes and Comments
Nicking of plasmid DNA	Overincubation during the alkaline lysis step. Incubation of cell suspension with Lysis Solution and Alkaline Protease should not exceed 5 minutes.
Poor results with automated fluorescent sequencing	Too little DNA was added to the sequencing reaction. Plasmid concentration not accurately quantitated. Use agarose gel/ethidium bromide quantitation.
	Wrong dilution buffer used with ABI PRISM® BigDye® sequencing chemistry. Use dilution buffer recommended in Section 4.C.
	TE buffer was used for DNA elution. Repurify plasmid DNA and elute in Nuclease-Free Water.
No restriction digestion	Concentration of restriction enzyme, length of digestion need to be increased. Increase the amount of restriction enzyme or the length of incubation time. Digest at suggested temperature and in the optimal buffer for the restriction enzyme used. Keep the volume of the miniprep DNA to 10% or less of reaction volume.
DNA yields on gel look low compared to spectrophotometer readings	Traces of contaminants may be present in the eluted DNA, which inflate the spectrophotometer readings. Use agarose gel/ethidium bromide quantitation.
Clogging of some wells in Lysate Clearing Plate	Too many bacterial cells processed per well. Use a maximum cell density of 4 $O.D._{600}$. Grow cells in 1–1.2ml of nonenriched medium (i.e., LB medium). Alternatively, process smaller culture volumes. Increase vacuum to 20 inches of Hg. Extend vacuum time by 10 minutes.

6. Composition of Buffers and Solutions

LB medium

10g	casein peptone
5g	yeast extract
5g	NaCl
15g	agar (for plates only)

Dissolve in 1L of distilled water. Autoclave and cool to 55°C before adding antibiotic. **Note:** For LB liquid medium, do not add agar.

10X TE buffer

100mM	Tris-HCl (pH 7.5)
10mM	EDTA

Terrific Broth

12g	Bacto® tryptone
24g	yeast extract
2.31g	KH_2PO_4
12.54g	K_2HPO_4

Add Bacto® tryptone and yeast extract to 900ml deionized water; sterilize by autoclaving. Combine salts in 100ml deionized water; autoclave to sterilize, then add 100ml to the broth.

Wizard® SV 96 Cell Lysis Solution

0.2M	NaOH
1%	SDS

Wizard® SV 96 Neutralization Solution

4.09M	guanidine hydrochloride
0.759M	potassium acetate
2.12M	glacial acetic acid

Final pH is approximately 4.2.

Wizard® SV 96 Resuspension Solution

50mM	Tris-HCl (pH 7.5)
10mM	EDTA
100µg/ml	RNase A

Wizard® SV 96 Wash Solution

162.8mM	potassium acetate
22.6mM	Tris-HCl (pH 7.5)
0.109mM	EDTA (pH 8.0)

Add 95% ethanol as described in Section 3: Add 170ml to the 1 × 96 well system or add 630ml to the 5 × 96 well system. Final concentrations will be approximately 60% ethanol, 60mM potassium acetate, 8.3mM Tris-HCl and 40µM EDTA.

7. Related Products

Product	Size	Cat.#
Alkaline Protease Solution*	3ml	A1441
Wizard® SV 96 Cell Resuspension Solution*	500ml	A7113
Wizard® SV 96 Cell Lysis Solution*	500ml	A7123
Wizard® SV 96 Neutralization Solution*	500ml	A1481
Wizard® SV 96 Wash Solution	370ml	A1318
Wizard® SV 96 Binding Plates*	10 pack	A2271
Wizard® *Plus* SV Minipreps DNA Purification System*	50 preps	A1330
Wizard® *Plus* SV Minipreps DNA Purification System* + Vacuum Adapters	50 preps	A1340
Wizard® *Plus* SV Minipreps DNA Purification System*	250 preps	A1460
Wizard® *Plus* SV Minipreps DNA Purification System* + Vacuum Adapters	250 preps	A1470
SV Total RNA Isolation System*	50 preps	Z3100
PureYield™ Plasmid Midiprep System	25 preps	A2492
	100 preps	A2495

*For Laboratory Use.

Product	Size	Cat.#
Wizard® SV 96 Lysate Clearing Plates*	10 pack	A2241
Vac-Man® Laboratory Vacuum Manifold	20-sample capacity	A7231
	2-sample capacity	A7660

*For Laboratory Use.

8. References

1. Kahn, M. *et al.* (1979) Plasmid cloning vehicles derived from plasmids *Col*E1, F, R6K, and RK2. *Meth. Enzymol.* **68**, 268–80.

2. Guntelberg, A.V. and Otteson, M. (1954) *Compt. Rend. Trav. Lab. Carlsberg* **29**, 36.

3. Aehle, W. *et al.* (1993) Rational protein engineering and industrial application: Structure prediction by homology and rational design of protein-variants with improved 'washing performance' – the alkaline protease from *Bacillus alcalophilus*. *J. Biotechnol.* **28**, 31–40.

4. van der Osten, C. *et al.* (1993) Protein engineering of subtilisins to improve stability in detergent formulations. *J. Biotechnol.* **28**, 55–66.

[a]U.S. Pat. No. 5,981,235, Australian Pat. No. 729932, European Pat. No. 0 918 877, Canadian Pat. No. 2,260,940 and other patents pending.

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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.

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Part# TB272 Printed in USA.

Revised 3/09