



Automated Wizard® SV 96 Plasmid DNA Purification System Protocol

Automated Protocol #EP002

DESCRIPTION OF THE LABORATORY ROBOTICS METHODS WITH PRODUCTS A2250 AND A2255.

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 Automated Protocol.

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Note: All Promega Technical Bulletins are available at: www.promega.com/tbs/

1. Description

This describes automation of the Wizard® SV 96 Plasmid DNA Purification System^(a). Specific instructions are provided for the Beckman Coulter Biomek® 2000 and Biomek® FX, Eppendorf epMotion® 5075 VAC and Hamilton MicroLab® STAR automated liquid-handling workstations. General automation guidelines are provided

for adaptation to other liquid-handling platforms. For information about obtaining validated methods, please see www.promega.com/automethods/. For troubleshooting chemistry issues, please refer to the *Wizard® SV 96 Plasmid DNA Purification System Technical Bulletin #TB272*.

2. Product Components

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

For Laboratory Use. Each system contains sufficient reagents for 1 × 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 5 × 96 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

3. Before You Begin

Materials to Be Supplied by the User

- LB agar plates containing antibiotic culture medium
- 95% ethanol
- liquid culture medium containing antibiotic
- centrifuge capable of 1,500 × *g*, fitted with 96-well deep-well plate adapter (e.g., tabletop model or Beckman Coulter J2HC model 362701 centrifuge)
- vacuum pump capable of 15–20 inches of Hg (e.g., Fisher Cat.# 01-092-29; Biomek® workstations only)
- vacuum tubing (Biomek® workstations only)

3. Before You Begin (continued)

Preparation of Materials

If you are using frozen cell pellets, allow the cell pellets to thaw at least 10–15 minutes at room temperature before processing.

Prior to beginning the procedure with a new Wizard® SV 96 Plasmid DNA Purification System, dilute the Wizard® SV 96 Wash Solution as follows:

For the 1 × 96 system (Cat.# A2250), add 170ml of 95% ethanol to the bottle of Wash Solution for a final volume of 270ml. For the 5 × 96 system (Cat.# A2255), add 630ml of 95% ethanol to the Wash Solution for a final volume of 1,000ml per bottle. Label the bottles to indicate that ethanol has been added.

4. Automated Processing Requirements for the Biomek® 2000 Workstation

4.A. Instrumentation Requirements for the Biomek® 2000 Workstation

The following is a list of Beckman Coulter parts and their corresponding part numbers that are required to automate the Wizard® SV 96 Plasmid DNA Purification System on a Biomek® 2000.

Part Description	Beckman Coulter Part Number
Biomek® 2000 Workstation, 50/60 Hz, 100–120V	609000
Biomek® 2000 Controller NT	609875
IBM Monitor	974571
BioWorks™ 3.2 for Beckman Coulter Computer	609983
Biomek® 2000 Right Side Module	609047
Gripper Tool System	609001
Eight Channel Wash Tool	609027
Wash Unit with Automatic 6-Port Valve	609056
MP200 Eight-Tip Pipette Tool	609025
Pipette Tip Rack Holder (2)	609121
Gray Labware Holder (4)	609120
Neoprene Gasket Kit, Collar (5 pieces)	609846
Labware Collar Holders (2)	609736
Vacuum Valve Unit	609005
Vacuum Filtration Manifold Base	609670
36mm Vacuum Collar	609597
65mm Vacuum Collar with Spacer Plate and Reservoir Frame	609803
Vacuum Regulator	609674
Tubing Kit, Filtration System	609676
Tubing Kit, Wash Unit	609687
Heavy-Duty Bottle, HDPP 4L	975796
Cap, Filling/Venting 1/2" Fittings	975797
Spacer Filter Plate	390792
Reservoir Frame	372795

4.B. Labware Requirements for the Biomek® 2000 Workstation

Part Description	Ordering Information
Binding Plate	Provided in Wizard® SV 96 Plasmid DNA Purification System
SV 96 Clearing Plate	Provided in Wizard® SV 96 Plasmid DNA Purification System
Elution Plate	Provided in Wizard® SV 96 Plasmid DNA Purification System
96-well 2ml square deep-well plate or comparable	Provided in Wizard® SV 96 Plasmid DNA Purification System
Biomek® P250 Tips (2 racks)	Beckman Coulter Part# 372654
Quarter Reservoir (3)	Beckman Coulter Part# 372790
Half Reservoir (1)	Beckman Coulter Part# 372786

4.C. Biomek® 2000 Workstation Initial Deck Configuration

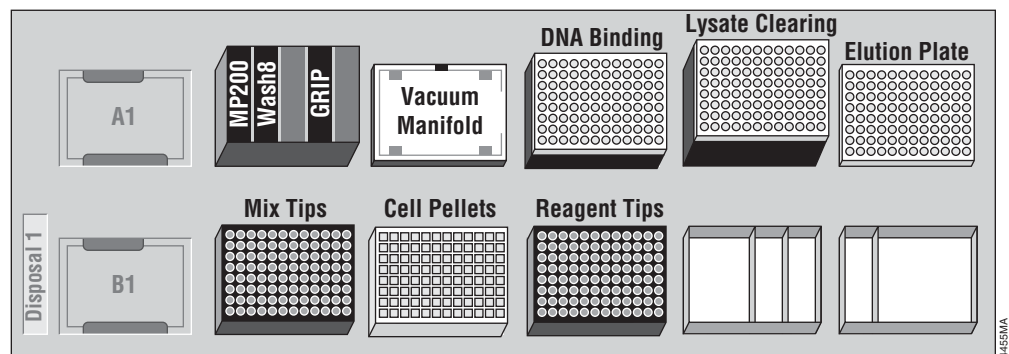


Figure 1. Biomek® 2000 Workstation Initial Deck Configuration.

Position A1	Empty
Position A2	Tool rack containing MP200, Wash 8 and Gripper tools
Position A3	Vacuum Filtration Manifold Base, Spacer Filter Plate, Spacer Plate
Position A4	Labware Collar holder, 36mm vacuum collar, Binding Plate
Position A5	Labware Collar holder, 65mm vacuum collar, Lysate Clearing Plate
Position A6	Labware Holder, Elution Plate
Position B1	Empty
Position B2	Pipette Tip rack holder, Biomek® P250 tips
Position B3	Labware holder, 96-Well 2ml Deep Well Plate containing cell pellets
Position B4	Pipette Tip rack holder, Biomek® P250 tips
Position B5	Labware holder, reservoir frame: half reservoir, 2 quarter reservoirs
Position B6	Labware holder, reservoir frame: quarter reservoir



Figure 2. Picture of position A3 setup on the Beckman Coulter Biomek® 2000 Workstation. To optimize the Elution Plate height and ensure the filter plate nozzles are just within the wells of the Elution Plate, two Beckman Coulter spacers are required: a Spacer Filter Plate and Spacer Plate. The clear plastic Spacer Plate is placed on the white Spacer Filter Plate. The two are placed in the Vacuum Filtration Manifold Base.

4.D. Reagent Dispense Volumes for the Biomek® 2000 Workstation

Prior to beginning the run, the following Wizard® SV 96 Plasmid DNA Purification System reagents need to be dispensed appropriately on the deck of the Biomek® 2000 workstation according to the initial configuration. Reagent volumes required are dependent on the number of samples processed. The automated method provides specific instructions in green text for reagent volumes to be dispensed at each position.

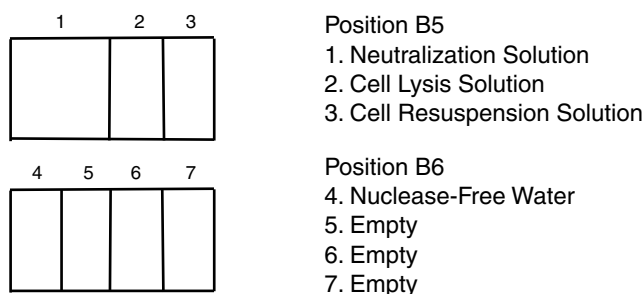


Figure 3. Reagent configuration for the Wizard® SV 96 Plasmid DNA Purification System.

4.E. Biomek® 2000 Workstation Specific Pre-Run Recommendations

Before running, the method must be imported into the BioWorks™ Software. Please follow the instructions provided in the document *Importing Beckman Coulter Biomek® 2000 Methods*.

(www.promega.com/automethods/beckman/biomek2000/default.asp)

Defining Sample Number for Processing

This method provides a simple way to modify the number of samples processed during the run. The number of samples processed can be changed from the default 96 samples/run to any number of samples divisible by 8. Therefore, runs of 8, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88 or 96 samples can be performed. To change the number of samples processed in each run, the filter plate pattern and the source plate pattern need to be redefined.

Defining Filter Plate Pattern

In the method edit screen of the BioWorks™ software for the “B2K SV 96 single” method, select EDIT in the menu bar. In the EDIT menu bar, select “Patterns.”

This will bring up the “EDIT Global Window.” In this window, highlight the SV 96 filter plate. This selection will activate a number of buttons in the window. Choose the EDIT button. This will bring up another window that is titled “SV 96 filter plate.” On the right side of the window is a small box labeled “Edit Pattern.” In the “Edit Pattern” box, select the “Allow Changes” option. Choose the CLEAR button at the bottom of the window. This clears the selection of wells being processed. Hold the cursor over the representation of the SV 96 filter plate and select the wells to be processed. Selected wells will turn blue. Once the wells are selected, click OK. You will return to the “EDIT Global Window.”

Defining Source Plate Pattern

Return to the “EDIT Global Window.” Highlight the Source Plate. This selection will activate a number of buttons in the window. Choose the EDIT button. This will bring up another window that is titled “Source Plate.” On the right side of the window is a small box labeled “Edit Pattern.” In this Edit Pattern box, select the “Allow Changes” option. Then choose the CLEAR button. This will clear the selection of wells being processed. Hold the cursor over the representation of the filter plate and select the wells corresponding to the number of samples being processed. Selected wells will turn blue. Once the wells are selected, click OK. You will return to the “EDIT Global Window.” Close the “EDIT Global Window” to return to the method edit screen.

5. Automated Processing Requirements for the Biomek® FX Workstation

5.A. Instrumentation Requirements for the Biomek® FX Workstation

Part Description	Quantity	Beckman Coulter Part Number
Minimum: Biomek® FX		
Software version 2.1		719349
96-channel POD	1	719368
Minimum number of Labware		
Positions by 1 POD	16	719357
Tip Loader	1	719356
Trash ALP	Optional	719347
Tip Wash ALP	Optional	719363
SPE ALP	1	717632
Holder ALP (for SPE)	1	719229
Vacuum Valve Unit	1	609005
Vacuum Filtration Manifold Base	1	609670
65mm Collar with Filter		
Reservoir Holder	1	609803
Tubing Kit, Filtration System	1	609676
Heavy-Duty Bottle, HDPP 4L	1	975796
Cap, Filling/Venting 1/2” Fittings	1	975797

5.B. Labware Requirements for the Biomek® FX Workstation

Part Description	Quantity	Ordering Information
96-well, Deep-Well, V-Bottom 2.2ml Plate	1	Promega Cat.# V6781
96-well Deep-Well Plate	1	Provided with Wizard® SV 96 Plasmid DNA Purification System
Binding Plate	1	Provided with Wizard® SV 96 Plasmid DNA Purification System
Wizard® SV 96 Lysate Clearing Plate	1	Provided with Wizard® SV 96 Plasmid DNA Purification System
Biomek® AP96 P250 tips	5	Beckman Coulter Part# 717251

5.C. Biomek® FX Workstation Initial Deck Configuration

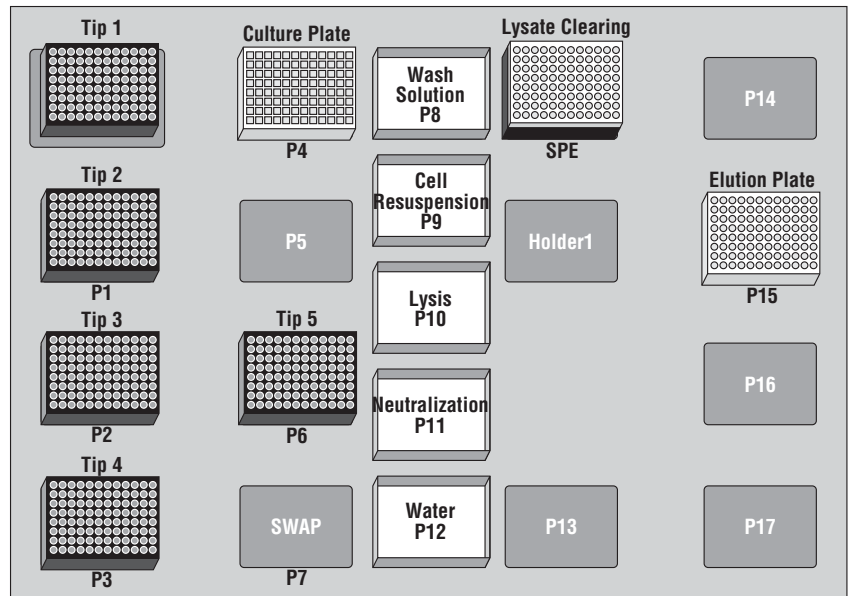


Figure 4. Biomek® FX initial deck configuration. This is an example of the Wizard® SV 96 Plasmid DNA Purification deck layout on a Biomek® FX Workstation. Your specific deck layout may be different, depending on your Biomek® FX Workstation configuration.

ALP Name	Equipment
Tip Loader	Biomek® AP96 P250 tips
P1	Biomek® AP96 P250 tips
P2	Biomek® AP96 P250 tips
P3	Biomek® AP96 P250 tips
P4	96-Well 2ml Deep-Well Plate containing bacterial cell pellets
P5	Empty
P6	Biomek® AP96 P250 tips
P7	Swap spot (empty)
P8	Upside-down tip box lid containing Wash Solution
P9	Upside-down tip box lid containing Cell Resuspension Solution
P10	Upside-down tip box lid containing Lysis Solution
P11	Upside-down tip box lid containing Neutralization Solution
P12	Upside-down tip box lid containing water
P13	Empty
P14	Empty
P15	Empty 2.2ml Deep-Well, V-bottom Plate for elution
P16	Empty
P17	Empty
SPE ALP	Beckman Coulter Vacuum manifold base, Filter Reservoir holder, Binding Plate, 65mm collar, SV 96 Lysate Clearing Plate
Holder ALP	Empty, used for vacuum manifold assembly

5.D. Biomek® FX Workstation Specific Pre-Run Recommendations

Beckman Coulter has developed software wizards for automated purification of plasmid DNA using the Wizard® SV 96 Plasmid Purification Systems and the Biomek® FX Workstation. Specific hardware and/or labware may be slightly different than described here. For more information, contact your local Beckman Coulter sales representative or visit Beckman Coulter's eLabnotebook™ at: <http://beckman.com/resourcecenter/elabnotebook/BasicResearchTargetID/NucleicAcidPrep/PlasmidPurification/index.asp>

The Biomek® FX Laboratory Workstation allows users the flexibility to configure the robot's deck according to need. Because of this, the deck used for writing a Biomek® FX Workstation method may differ from an end-user's deck. Therefore, it is generally necessary to map an imported method onto an end-user's deck configuration. To map an imported method onto your deck, please follow the instructions provided in the document *Biomek® FX Deck Mapping*. (www.promega.com/automethods/beckman/biomekfx/default.asp)

6. Automated Processing Requirements for the Eppendorf epMotion® 5075 VAC Workstation

6.A. Instrumentation Requirements for the epMotion® 5075 VAC Workstation

The following is a list of Eppendorf parts and their corresponding part numbers that are required to automate the Wizard® SV 96 Plasmid DNA Purification System on an epMotion® 5075 VAC Workstation.

Part Description	Quantity	Eppendorf Part Number
epMotion® 5075 VAC workstation, Gripper and Waste Tub	1	5075 000.016
TM 1000-8, 8-channel dispensing tool	1	5280 000.258
Reservoir Rack (100ml reagent reservoirs)	1	5075 754.002
55mm Height adapters	2	5075 752.000
Vac Frame	1	5075 784.009
Vac Frame 2	1	5075 784.005

6.B. Labware Requirements for the epMotion® 5075 VAC Workstation

Part Description	Quantity	Eppendorf Part Number
100ml epMotion Reservoirs	6	0030 126.513
1,000µl Filter Tips	4	0030 003.993

6.C. epMotion® 5075 VAC Workstation Initial Deck Configuration

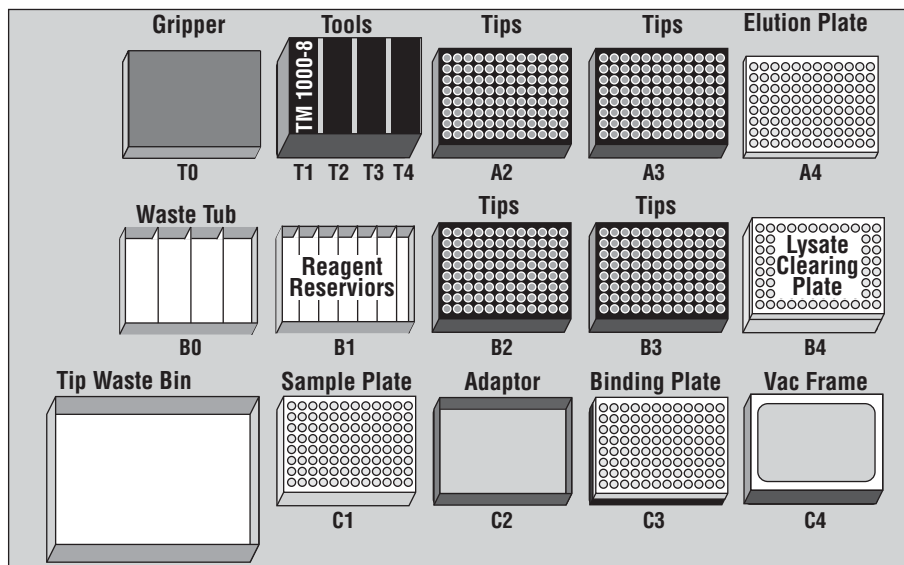


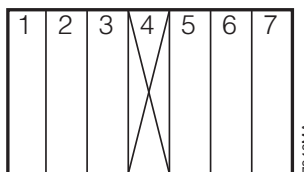
Figure 5. Initial configuration of epMotion® 5075 VAC Workstation.

Position T0	Gripper and Vacuum Lid
Position T1	TM1000-8 Dispensing Tool
Positions T2–T4	Empty
Position A2	1,000µl epTIPS Motion Filtered
Position A3	1,000µl epTIPS Motion Filtered
Position A4	Elution Plate
Position B0	Waste Tub with quarter wall separators
Position B1	6 × 100ml Reagent Reservoirs (see Section 6.D)
Position B2	1000µl epTIPS Motion Filtered
Position B3	1000µl epTIPS Motion Filtered
Vacuum	Empty 96-Well Deep-Well Plate (inside), Vac Frame 2, Lysate Clearing Plate (top)
Position C1	96-Well Deep-Well Plate containing samples
Position C2	55mm Height Adaptor
Position C3	55mm Height Adaptor, Binding Plate (top)
Position C4	Vac Frame

6.D. Reagent Dispense Volumes for the epMotion® 5075 VAC Workstation

Prior to beginning the run, reagents must be dispensed onto the deck of the epMotion® 5075 VAC Workstation. Place 6 Reagent Reservoirs on the epMotion® 5075 Workstation deck in the Reservoir Rack at position B1 and fill as follows:

1. 100ml Reservoir: 40ml Wizard® SV 96 Cell Resuspension Solution
2. 100ml Reservoir: 40ml Wizard® SV 96 Cell Lysis Solution
3. 100ml Reservoir: 84ml Wizard® SV 96 Neutralization Solution
4. Empty
5. 100ml Reservoir: 100ml Wizard® SV 96 Wash Solution (ethanol added)
6. 100ml Reservoir: 100ml Wizard® SV 96 Wash Solution (ethanol added)
7. 100ml Reservoir: 13ml Nuclease-Free Water



7. Automated Processing Requirements for the Hamilton MicroLab® STAR Workstation

7.A. Instrumentation Requirements for the MicroLab® STAR Workstation

The following is a list of Hamilton parts and their corresponding part numbers that are required to automate the Wizard® SV 96 Plasmid DNA Purification System on a MicroLab® STAR Workstation.

Part Description	Quantity	Hamilton Part Number
MicroLab® STAR workstation	1	173000-020
BVS Carrier	1	190033
BVS Carrier with height adaptors	1	190035
5-Position Tip Rack Carrier	1	182085
5-Position DWP Carrier for Reservoirs	1	182090
5-Position Plate Carrier for Standard Microplates	1	182365

7.B. Labware Requirements for the MicroLab® STAR Workstation

Part Description	Quantity	Part Number
300µl CO-RE Disposable Filter Tips in blue hanging rack	4	Hamilton Cat.# 235903
1,000µl CO-RE Disposable Filter Tips in blue hanging rack	1	Hamilton Cat.# 235905
Pyramid-Bottom Reservoir Plates	4	Promega Cat.# V6801
12-Column Deep Well Reservoir	1	Promega Cat.# V6791

7.C. MicroLab® STAR Workstation Initial Deck Configuration

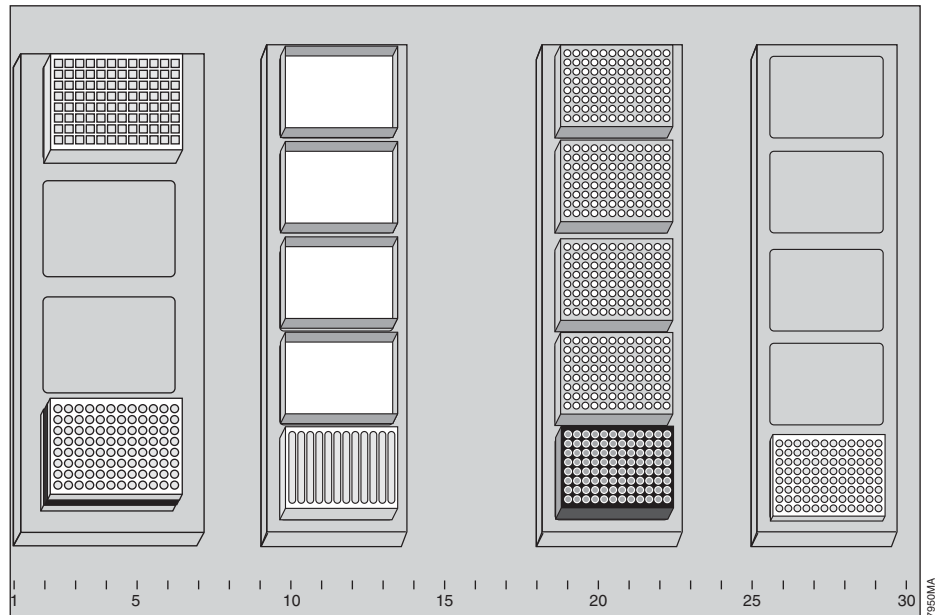


Figure 6. Initial configuration of MicroLab® STAR Workstation

- Grid 1** BVS Carrier
 - Site 1: 96-well, deep-well plate containing samples
 - Site 4: Binding Plate (inside), Height Adaptor, BVS cover, Lysis Clearing Plate (top)
- Grid 9** 5-Position DWP Carrier for Reservoirs
 - Site 1: Pyramid Bottom Reservoir Plate (Resuspension Solution)
 - Site 2: Pyramid Bottom Reservoir Plate (Cell Lysis Solution)
 - Site 3: Pyramid Bottom Reservoir Plate (Neutralization Solution)
 - Site 4: Pyramid Bottom Reservoir Plate (Wash Solution, ethanol added)
 - Site 5: 12-Column Deep Well Reservoir (Nuclease-Free Water in Column 1)
- Grid 18** 5-Position Tip Rack Carrier
 - Site 1: 300µl CO-RE Disposable Filter Tips in blue hanging rack
 - Site 2: 300µl CO-RE Disposable Filter Tips in blue hanging rack
 - Site 3: 300µl CO-RE Disposable Filter Tips in blue hanging rack
 - Site 4: 300µl CO-RE Disposable Filter Tips in blue hanging rack
 - Site 5: 1,000µl CO-RE Disposable Filter Tips in blue hanging rack
- Grid 25** 5-Position Plate Carrier for Standard Microplates
 - Site 5: 96-well Clear Elution Plate (provided with the Wizard® SV 96 Plasmid DNA Purification System)

7.D. Reagent Dispense Volumes for the MicroLab® STAR Workstation

Prior to beginning the run, reagents must be dispensed onto the deck of the MicroLab® STAR Workstation. Place four Pyramid Bottom Reservoir Plates on the MicroLab® STAR Workstation deck on the DWP Carrier at grid position 9, sites 1–4, and one 12-Column Deep-Well Reservoir at grid position 9, site 5, and fill as follows:

- Site 1: 40ml Wizard® SV 96 Cell Resuspension Solution
- Site 2: 40ml Wizard® SV 96 Cell Lysis Solution
- Site 3: 84ml Wizard® SV 96 Neutralization Solution
- Site 4: 220ml Wizard® SV 96 Wash Solution (ethanol added)
- Site 5: (Column 1) 13ml Nuclease-Free Water

8. Description of the Automated Wizard® SV 96 Plasmid DNA Purification System

This overview describes general liquid-handling steps required for automated Wizard® SV 96 Plasmid DNA Purification and can be adapted to a variety of automated liquid-handling robots. For additional information for adaptation to liquid-handling robots other than those referenced above, please see Section 9.

1. **Resuspension of Bacterial Cell Pellets.** Resuspension Solution (250µl) is added to each well of the 96-well 2ml Deep-Well Plate containing bacterial cell pellets and mixed by pipetting.
2. **Cell Lysis.** Lysis Solution (250µl) is added to each well of the 96-well 2ml Deep-Well Plate, and the samples are mixed by pipetting.
3. **System Pause.** The system pauses for 4.5 minutes to allow cell lysis.
4. **Neutralization.** Neutralization Solution (350µl) is added to each well of the 96-well Deep-Well Plate, and the samples are mixed.
5. **Transfer Lysate to Lysate Clearing Plate.** Cell lysate (950µl) is transferred from each well of the 96-well Deep-Well Plate to the Wizard® SV 96 Lysate Clearing Plate located on the vacuum manifold.
6. **Vacuum Transfer from Lysate Clearing Plate to Binding Plate.** A vacuum is applied for approximately 6 minutes to transfer the lysate through the Lysate Clearing Plate and onto the Binding Plate.
7. **Restack of Filtration Stack.** The Wizard® SV 96 Lysate Clearing Plate is removed and reassembled on the vacuum manifold for plasmid DNA capture from the Binding Plate.
8. **Binding Plasmid DNA to Binding Plate.** The vacuum is applied to pull the cell lysate through the Binding Plate. During this time, the plasmid DNA binds to the Binding Plate.
9. **Neutralization Solution Wash.** Neutralization Solution (200µl) is added to each well of the Binding Plate. The vacuum is applied to pull it through the Binding Plate.
10. **Wash Binding Plate.** Wash Solution (1ml) is dispensed across the Binding Plate. The vacuum is applied to allow the Wash Solution to pass through the Binding Plate, washing the plasmid DNA. This wash is repeated with another 1ml of Wash Solution.

11. **Drying of Binding Plate.** The vacuum is applied for 6 minutes to remove residual ethanol and dry the Binding Plate.
12. **Assembly for Elution.** The gripper disassembles the vacuum manifold stack by moving the Binding Plate and manifold collar to a holding position. The gripper then moves a 96-well, flat-bottom Elution Plate into the bottom of the vacuum manifold. The gripper then reassembles the vacuum manifold stack by moving the Binding Plate and collar back onto the vacuum manifold.
13. **Elution of Plasmid DNA from Binding Plate.** Nuclease-Free Water (100 μ l) is added to each well of the Binding Plate. The system pauses for one minute. The vacuum is applied, and the DNA is eluted from the Binding Plate into the Elution Plate.
14. **Method Ends.** Purified plasmid DNA has been eluted into the Elution Plate.

9. General Guidelines for Adaptation to Alternative Robotic Platforms

This method uses vacuum filtration of samples for binding, washing and elution. To ensure that sufficient pressure is applied, make sure that the vacuum pump you are using is set to pull a vacuum of 15–20 inches of Hg. Vacuum pressure less than 15 inches of Hg will result in reduced DNA yield and purity and may cause column clogging when processing lysates. Vacuum pressure greater than 20 inches of Hg may result in spraying; this increases the probability of sample cross-contamination.

(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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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.

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