

Automated Wizard® SV 96 Genomic DNA Purification System for Cell Culture Samples

Automated Protocol #EP018

DESCRIPTION OF THE AUTOMATED METHODS WITH PRODUCTS A2370 AND A2371
Please visit the web site to verify that you are using the most current version of this Automated Protocol.

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1. Description

This protocol describes automation of the Wizard® SV 96 Genomic DNA Purification System^(a) to purify genomic DNA from tissue culture cells. Specific instructions are provided for the following automated liquid-handling workstations: Beckman Coulter Biomek® 2000, Biomek® 3000, Biomek® FX and Eppendorf epMotion® 5075 VAC. Information about obtaining validated methods for these automated liquid-handling workstations is available at: www.promega.com/automethods/

General automation guidelines are provided for adaptation to other liquid-handling platforms. To troubleshoot chemistry issues please refer to the *Wizard® SV 96 Genomic DNA Purification System Technical Bulletin #TB303*.

2. Product Components and Storage Conditions

Product	Size	Cat.#
Wizard® SV 96 Genomic DNA Purification System	1 × 96 preps	A2370

Each system contains sufficient reagents for 96 isolations. Includes:

- 50ml Nuclei Lysis Solution
- 30ml 0.5M EDTA (pH 8.0)
- 50ml Wizard® SV Lysis Buffer
- 185ml Wizard® SV Wash Solution (concentrated)
- 1ml RNase A Solution (4mg/ml)
- 150ml Nuclease-Free Water
- 1 Binding Plate
- 1 96-Well Deep Well Plate
- 1 Protocol

Product	Size	Cat.#
Wizard® SV 96 Genomic DNA Purification System	4 × 96 preps	A2371

Each system contains sufficient reagents for 4 × 96 isolations. Includes:

- 2 × 50ml Nuclei Lysis Solution
- 30ml 0.5M EDTA (pH 8.0)
- 3 × 50ml Wizard® SV Lysis Buffer
- 2 × 370ml Wizard® SV Wash Solution (concentrated)
- 3 × 1ml RNase A Solution (4mg/ml)
- 2 × 150ml Nuclease-Free Water
- 4 Binding Plates
- 4 96-Well Deep Well Plates
- 1 Protocol

Storage Conditions: Store all components at 22–25°C.

3. Materials to Be Supplied by the User

- 1X phosphate-buffered saline (PBS), sterile
- 55°C water bath
- adhesive plate sealers
- **for Biomek® FX only:** Pyramid-Bottom Reservoir Plates (2)
(Promega Cat.# V6801)

4. Before You Begin

4.A. Preparation of Wizard® SV Wash Solution

Prepare the Wizard® SV Wash Solution prior to beginning the Wizard® SV 96 Genomic DNA Purification System protocol.

Add 95% ethanol to the Wizard® SV Wash Solution bottle as directed on the bottle label. Label the bottle to indicate that ethanol has been added. Seal well, and store at room temperature.

4.B. Preparation of Cell Culture Samples

Before placing cells on the deck of the robot, wash the cells once with 1X PBS. Make sure to remove PBS before placing cells on the deck of the robot for processing. Wizard® SV Lysis Buffer should be added to cells alone.

Use at least 1×10^4 cells and up to 5×10^6 cells per purification. The number of cells may need to be adjusted depending on cell type and function.

5. Biomek® 2000 Workstation Requirements

5.A. Method

1. Check instrument requirements for the Beckman Coulter Biomek® 2000 Wizard® SV 96 Genomic DNA cell culture sample method.

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

Part Description	Beckman Coulter Part Number
Biomek® 2000 workstation, 50/60 Hz, 100–120V	609000
Controller, Biomek® 2000, with BioWorks™ 3.2 for new systems	267653
Gripper kit	609001
Eight-channel wash tool	609027
Wash unit with automatic 6-port valve	609056
MP200 eight-channel pipetting tool	609025
Pipette tip rack holder, black (2 for single plate run)	609121
Labware holder, gray (3)	609120
Biomek® 2000 96-Filtration System for Promega Wizard® SV 96 Systems (with Vacuum Unit)	267693
Elution plate spacer	390792
Tubing kit, wash unit	609687

2. Check labware requirements for the Beckman Coulter Biomek® 2000 Wizard® SV 96 Genomic DNA cell culture sample method.

Part Description	Ordering Information
Reservoir holder	372795
Quarter reservoir (2)	372790
96-Well Deep Well Plate	Provided
Binding Plate	Provided
96-well flat-bottom cell culture plate	Not Provided
P250 Tips, Sterile, 250µl (2)	Beckman Cat.# 372655
or P250 Tips, Racked, Sterile, 250µl (2)	Axygen Cat.# BT-250-1-R-S

5.A. Method (continued)

3. Check initial deck configuration for the Beckman Coulter Biomek® 2000 Wizard® SV 96 Genomic DNA Cell Culture Sample Method. The volumes of reagents dispensed in the reservoir at position B4 are shown in Figure 2.

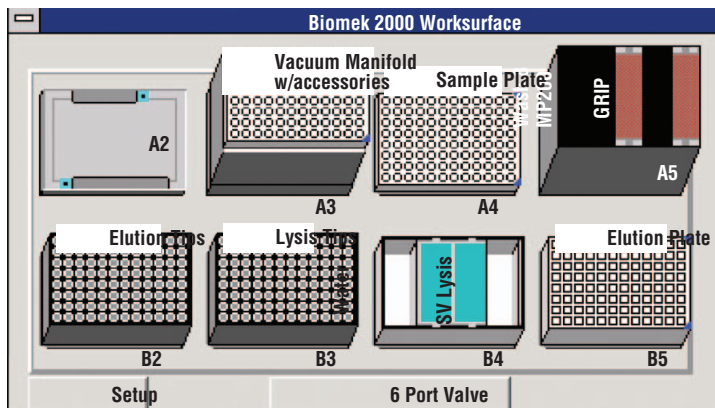
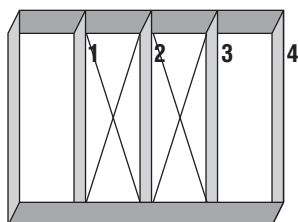


Figure 1. Biomek® 2000 initial deck configuration. Genomic DNA purification from cell culture samples.

Note: Side modules are not part of the initial deck configuration.

Position A2	Labware collar holder
Position A3	Vacuum filtration manifold base, elution plate spacer, 65mm collar, Binding Plate
Position A4	Labware holder, 96-well flat-bottom cell culture plate
Position A5	Tool rack containing eight-channel wash tool, MP200 eight-channel pipetting tool and Gripper kit
Position B2	Pipette tip rack holder, P250 tips
Position B3	Pipette tip rack holder, P250 tips
Position B4	Labware holder, reservoir holder, two quarter reservoirs
Position B5	Labware holder, 96-Well Deep Well Plate



1. 12ml of Nuclease-Free Water
2. Empty
3. Empty
4. 17.5ml of Wizard® SV Lysis Buffer

Valve 1 of the Biomek® Wash unit should be connected to a bottle with at least 250ml of Wizard® SV Wash Solution (ethanol added).

Figure 2. Reagent dispense volumes for the Biomek® 2000. Prior to beginning run, the reagents listed above need to be dispensed appropriately on the deck of the Biomek® 2000.

5.B. Pre-Run Recommendations

Before running the method, import the method into the BioWorks™ software. Please follow the instructions for “Importing Biomek® 2000 Methods” available online at: www.promega.com/automethods/beckman/biomek2000/

6. Biomek® 3000 Workstation Requirements

6.A. Method

1. Check instrument requirements for the Beckman Coulter Biomek® 3000 Wizard® SV 96 Genomic DNA cell culture sample method.

The following is a list of Beckman Coulter parts and their corresponding part numbers that are required to automate the Wizard® SV 96 Genomic DNA Purification System for cell culture samples on a Biomek® 3000 workstation.

Part Description	Beckman Coulter Part Number
Biomek® 3000 workstation, 50/60 Hz, 100–120V	986120
Biomek® automation controller XP and monitor with Biomek® system software	A16170
Gripper kit	A09053
Eight-channel wash tool	987370
Wash unit with automatic 6-port valve	609056
Left-side module	987264
MP200 pipetting tool	986146
Pipette tip rack holder, black (2)	391910
Labware holder, gray (3)	609120
Biomek® 3000 filtration system with vacuum valve unit	A15925
Collar (65mm) with spacer plate	609803
Elution plate spacer	390792

2. Check labware requirements for the Beckman Coulter Biomek® 3000 Wizard® SV 96 Genomic DNA cell culture sample method.

Part Description	Ordering Information
Reservoir holder	372795
Quarter reservoir (2)	372790
96-Well Deep Well Plate	Provided
Binding Plate	Provided
96-well flat-bottom cell culture plate	Not Provided
Biomek® AP96 P250 Tips, Presterile (2)	Beckman Cat.# 717252
or Biomek® FX Tips, 250µl, Racked, Presterilized (2)	Axygen Cat.# FX-250-1-R-S

6.A. Method (continued)

3. Check initial deck configuration for the Beckman Coulter Biomek® 3000 Wizard® SV 96 Genomic DNA cell culture sample method. The volumes of reagents dispensed in the reservoir at position B3 are shown in Figure 4.

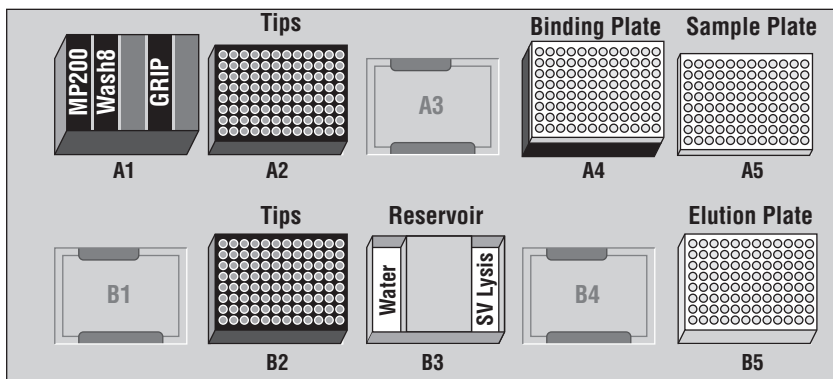
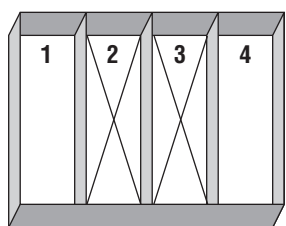


Figure 3. Biomek® 3000 initial deck configuration. Genomic DNA purification from cell culture samples.

Position A1	Tool rack containing MP200 pipetting tool, eight-channel wash tool and Gripper kit
Position A2	Pipette tip rack holder, P250 tips
Position A3	Empty
Position A4	Biomek® 3000 filtration system with vacuum valve unit, elution plate spacer, collar (65mm), Binding Plate
Position A5	Labware holder, 96-well flat-bottom cell culture plate
Position B1	Empty
Position B2	Pipette tip rack holder, P250 tips
Position B3	Labware holder, reservoir holder, 2 quarter reservoirs
Position B4	Empty
Position B5	Labware holder, 96-Well Deep Well Plate



1. 27ml of Nuclease-Free Water
 2. Empty
 3. Empty
 4. 20ml of Wizard® SV Lysis Buffer
- Valve 1 of the Biomek® Wash unit should be connected to a bottle of at least 250ml of Wizard® SV Wash Solution (ethanol added).

Figure 4. Reagent dispense volumes for the Biomek® 3000. Prior to beginning run, the reagents listed above need to be dispensed appropriately on the deck of the Biomek® 3000.

7. Biomek® FX Workstation Requirements

7.A. Method

The cell culture sample method will process one or two plates of cultured cells per method run. The instrument requirements for the one- and two-plate runs are identical. However, labware requirements and the starting deck layout for a one-plate or two-plate run are different (see labware requirements list and starting deck layout figures below).

1. Check instrument requirements for the Beckman Coulter Biomek® FX Wizard® SV 96 Genomic DNA cell culture sample method.

Part Description	Beckman Coulter Part Number
Minimum: Biomek® FX software v2.1	Contact Beckman Coulter
96-channel POD	Contact Beckman Coulter
Minimum number of labware positions by 1 POD (10)	Contact Beckman Coulter
Tip loader	719356
Biomek® FX filtration system (single plate) with vacuum valve unit	719400
Elution plate spacer	390792

2. Check labware requirements for the Beckman Coulter Biomek® FX Wizard® SV 96 Genomic DNA cell culture sample method.

Part Description	Quantity	Ordering Information
Pyramid-bottom reservoir plates	3 for one-plate method 4 for two-plate method	Promega Cat.# V6801
96-well flat-bottom cell culture plate	1 for one-plate method 2 for two-plate method	Not Provided
96-Well Deep Well Plate	1 for one-plate method 2 for two-plate method	Provided
Binding Plate	1 for one-plate method 2 for two-plate method	Provided
Biomek® AP96 P250 Tips, Presterile (2) or Biomek® FX Tips, 250µl, Racked, Presterilized (2)		Beckman Cat.# 717252 Axygen Cat.# FX-250-1-R-S

7.A. Method (continued)

3. Check initial deck configuration for Beckman Coulter Biomek® FX Wizard® SV 96 Genomic DNA cell culture sample method.

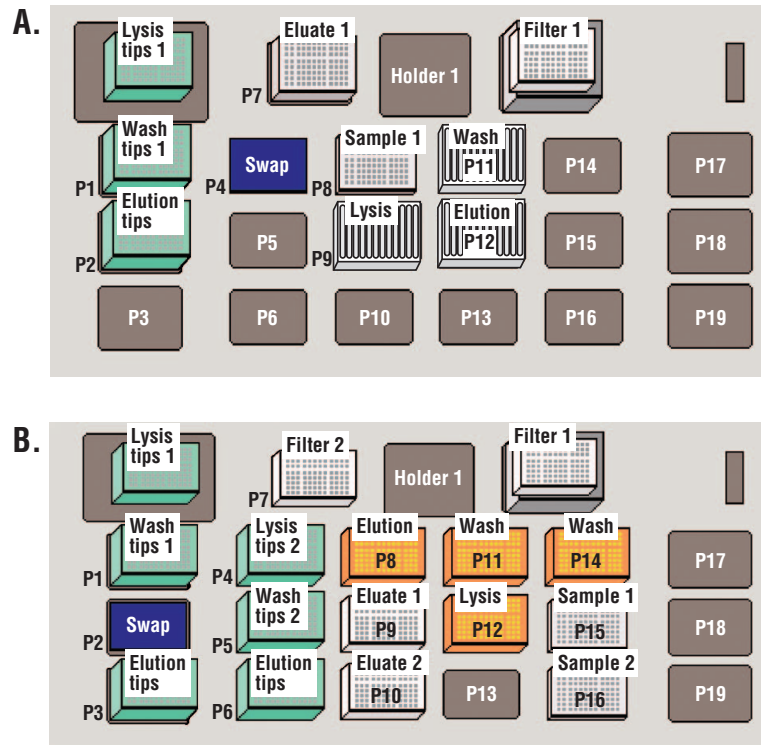


Figure 5. Biomek® FX deck layout. Examples of starting deck layouts for Wizard® SV 96 Genomic DNA Purification on a Biomek® FX. Your specific deck layout may be different depending on your Biomek® FX configuration.

A. Starting Deck Layout for One-Plate Cell Culture Sample Method.

ALP Name	Part Sitting on ALP
Tip Loader	P250 Biomek® FX tips
SPE ALP	Biomek® FX filtration system (single plate) with vacuum valve unit, elution plate spacer, Binding Plate
P1	P250 Biomek® FX tips
P2	P250 Biomek® FX tips
P4	Swap spot
P7	96-Well Deep Well Plate
P8	96-well flat-bottom cell culture plate
P9	Pyramid-bottom reservoir plate containing Wizard® SV Lysis Buffer
P11	Pyramid-bottom reservoir plate containing Wizard® SV Wash Solution (ethanol added)
P12	Pyramid-bottom reservoir plate containing Nuclease-Free Water

B. Starting Deck Layout for Two-Plate Cell Culture Sample Method.

ALP Name	Part Sitting on ALP
Tip Loader	P250 Biomek® FX tips
SPE ALP	Biomek® FX filtration system (single plate) with vacuum valve unit, elution plate spacer, Binding Plate
P1	P250 Biomek® FX tips
P2	Swap spot
P3	P250 Biomek® FX tips
P4	P250 Biomek® FX tips

Panel B description is continued on next page.

B. Starting Deck Layout for Two-Plate Cell Culture Sample Method. (continued)

ALP Name	Part Sitting on ALP
P5	P250 Biomek® FX tips
P6	P250 Biomek® FX tips
P7	Binding Plate for second sample-plate purification
P8	Pyramid-bottom reservoir plate containing Nuclease-Free Water
P9	96-Well Deep Well Plate #1
P10	96-Well Deep Well Plate #2
P11	Pyramid bottom reservoir plate containing Wizard® SV Wash Solution (ethanol added)
P12	Pyramid bottom reservoir plate containing Wizard® SV Lysis Buffer
P14	Pyramid bottom reservoir plate containing Wizard® SV Wash Solution (ethanol added)
P15	96-well flat-bottom cell culture plate #1
P16	96-well flat-bottom cell culture plate #2

Reagent Dispense Volumes for the Biomek® FX.

ALP Name	Part Sitting on ALP
One-Plate Cell Culture Sample Method	
P9 Reservoir	25ml Wizard® SV Lysis Buffer
P11 Reservoir	250ml Wizard® SV Wash Solution (ethanol added)
P12 Reservoir	30ml Nuclease-Free Water
Two-Plate Cell Culture Sample Method	
P12 Reservoir	40ml Wizard® SV Lysis Buffer
P11 Reservoir	250ml Wizard® SV Wash Solution (ethanol added)
P14 Reservoir	250ml Wizard® SV Wash Solution (ethanol added)
P8 Reservoir	50ml Nuclease-Free Water

7.B. Pre-Run Recommendations

The Biomek® FX automated platform allows users the flexibility to configure the robot's deck configuration according to need. Because of this flexibility in deck configuration, it is likely that the deck used for writing a Biomek® FX method will differ from an end-user's deck. Therefore, it will be generally necessary to map an imported method onto an end-user's deck configuration. Follow the instructions for "Biomek® FX Deck Mapping" available online at:

www.promega.com/automethods/beckman/biomekfx/

Prior to the first run of the Wizard® SV 96 Genomic DNA Purification method on the Biomek® FX, check all gripper moves to ensure that the vacuum manifold disassembly and reassembly for elution is correct. Our experience indicates that proper configuration of the gripper moves is essential to ensure success of Wizard® SV 96 methods on the Biomek® FX. Not performing the gripper evaluation may result in failure of vacuum manifold disassembly and reassemble and may damage your Biomek® FX instrument.

Follow the instructions for "Evaluation of Biomek® FX SV 96 Method Gripper Moves" available online at: **www.promega.com/automethods/beckman/biomekfx/**

"Evaluation of Biomek® FX SV 96 Method Gripper Moves" requires the Biomek® FX grip test method. Please inquire about this method at:

www.promega.com/automethods/beckman/biomekfx/

8. *epMotion*[®] 5075 VAC Workstation Requirements

8.A. Method

1. Check instrument requirements for the Eppendorf *epMotion*[®] 5075 VAC Wizard[®] SV 96 Genomic DNA cell culture sample method.

The following is a list of Eppendorf parts and their corresponding part number that are required to automate the Wizard[®] SV 96 Genomic DNA Purification System for cell culture samples on an *epMotion*[®] 5075 VAC workstation.

Part Description	Eppendorf Part Number
<i>epMotion</i> [®] 5075 VAC workstation, gripper and waste tub	5075 000.016
TM 300-8, 8-channel dispensing tool	5280 000.231
TM 1000-8, 8-channel dispensing tool	5280 000.258
Reservoir rack (100ml reagent reservoirs)	5075 754.002
85mm height adapter	5075 751.003
Vac holder	5075 778.009
Vac frame 2	Contact Eppendorf

2. Check labware requirements for the Eppendorf *epMotion*[®] 5075 VAC Wizard[®] SV 96 Genomic DNA cell culture sample method.

Part Description	Eppendorf Part Number
100ml <i>epMotion</i> [®] reservoirs (5)	0030 126.513
300µl epTIPS motion filter tips (1)	0030 003.977
1,000µl epTIPS motion filter tips (1)	0030 003.993
96-Well Deep Well Plate	Provided
Binding Plate	Provided
96-well flat-bottom cell culture plate	Not Provided

3. Check initial deck configuration for the Eppendorf *epMotion*[®] 5075 VAC Wizard[®] SV 96 Genomic DNA cell culture sample method. The volumes of reagents dispensed in the reservoirs at position A3 are shown in Figure 7.

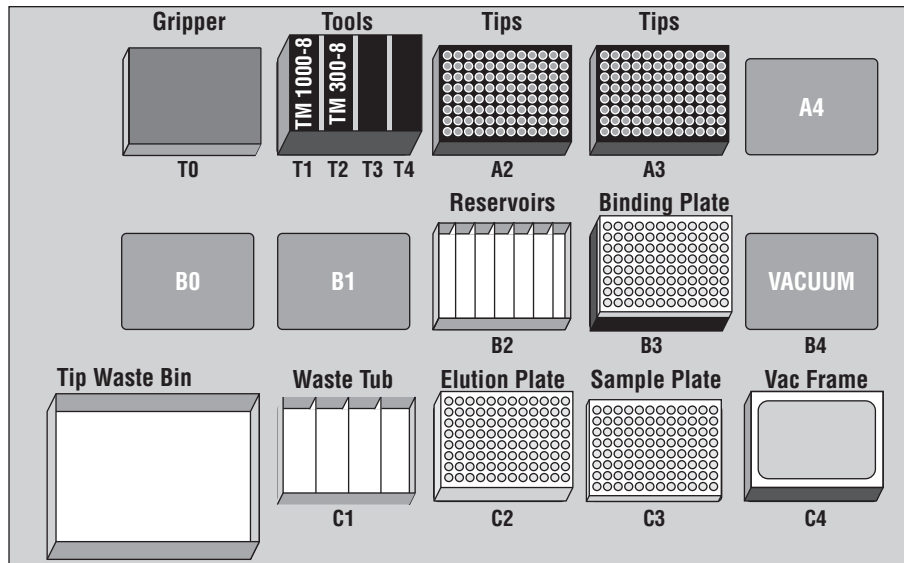
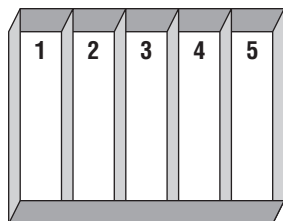


Figure 6. *epMotion*[®] 5075 VAC initial deck configuration. Genomic DNA purification from cell culture samples.

Position T0	Gripper
Position T1	TM1000-8, 8-channel dispensing tool
Position T2	TM 300-8, 8-channel dispensing tool
Position T3	Empty
Position T4	Empty
Position A2	1000µl epTIPS motion filter tips
Position A3	300µl epTIPS motion filter tips
Position A4	Empty
Position B0	Empty
Position B1	Empty
Position B2	Reservoir rack with 5 reagent reservoirs
Position B3	Binding Plate atop 85mm height adapter
Vacuum	Empty
Position C1	Waste tub with quarter wall separators
Position C2	96-Well Deep Well Plate
Position C3	96-well cell culture plate
Position C4	Vac frame 2 atop Vac holder



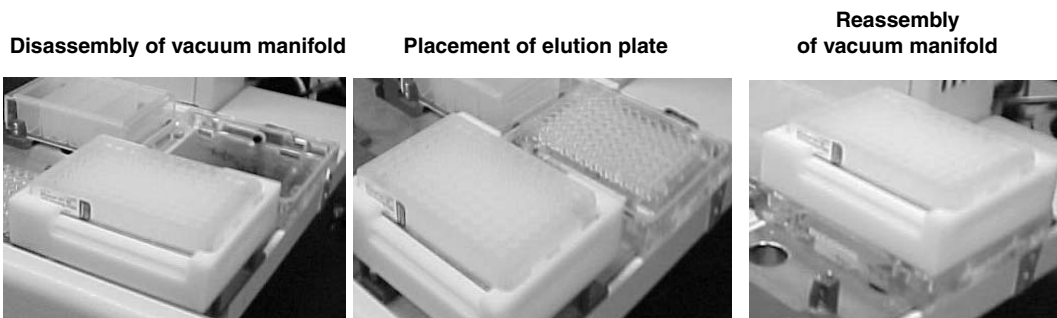
1. 100ml reservoir: 20ml of Wizard[®] SV Lysis Buffer
2. 100ml reservoir: 100ml of Wizard[®] SV Wash Solution (ethanol added)
3. 100ml reservoir: 100ml of Wizard[®] SV Wash Solution (ethanol added)
4. 100ml reservoir: 100ml of Wizard[®] SV Wash Solution (ethanol added)
5. 100ml reservoir: 30ml of Nuclease-Free Water

Figure 7. Reagent dispense volumes for the *epMotion*[®] 5075 VAC workstation. Prior to beginning run, the reagents listed above need to be dispensed appropriately on the deck of the *epMotion*[®] 5075 VAC workstation.

9. Description of Automated Wizard® SV 96 Genomic DNA Purification

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

1. **Lyse Cells.** Transfer 150µl of Wizard® SV Lysis Buffer from a reservoir to each well of the 96-well sample plate. Mix by pipetting.
2. **Transfer Cell Lysates.** Transfer the cell lysate contained in the sample plate to the Binding Plate sitting on top of the vacuum manifold apparatus.
3. **Bind Genomic DNA to the Binding Plate.** Once the cell lysates have been transferred to the Binding Plate, apply the vacuum and cell lysate is pulled through the Binding Plate by vacuum. Vacuum time may vary depending on sample type. During this vacuum step, genomic DNA binds to the Binding Plate.
4. **Wash Binding Plate.** Dispense 500µl of Wizard® SV Wash Solution (ethanol added) to each well of the Binding Plate. Apply the vacuum, and the wash solution is pulled through the Binding Plate. This step is repeated for a total volume of 2.5ml of Wizard® SV Wash Solution per well.
5. **Dry to Remove Residual Alcohol.** Apply the vacuum for 6–10 minutes to remove any residual ethanol from the Binding Plate.
6. **Prepare for Elution.** After the final vacuum step there is a one-minute pause to allow complete vacuum ventilation before disassembly and reassembly for the final elution step. A gripper tool disassembles the vacuum manifold stack by removing the Binding Plate from the vacuum manifold to a holding position. The gripper then moves the deep-well elution plate into the vacuum manifold. The gripper then reassembles the vacuum manifold stack by moving the Binding Plate back onto the vacuum manifold on the top of the elution plate.



Example of vacuum manifold stack disassembly, placement of elution plate and reassembly of vacuum manifold stack to elute purified DNA on the Biomek® 2000 workstation.

7. **Elute Purified Genomic DNA.** Transfer 200µl of Nuclease-Free Water from the reservoir to each well of the Binding Plate. Apply the vacuum, and the Nuclease-Free Water is pulled through the Binding Plate, eluting the genomic DNA into the elution plate. An elution volume of 200µl is recommended for optimal DNA yield from tissue culture cells. However, the elution volume may need to be optimized depending on amount of sample being processed and desired final concentration of eluted genomic DNA. Smaller elution volumes will increase concentration but may decrease the total DNA yield.
8. **Method Ends.** Purified genomic DNA has been eluted into the 96-Well Deep Well Plate sitting in the vacuum manifold. Dispose of the Binding Plate after use.

10. General Guidelines for Adaptation to Alternative Robotic Platforms

This method uses vacuum filtration to bind, wash and elute DNA samples. Make sure that your vacuum pump is set to pull a vacuum of 15–20 inches Hg. Vacuum pressure less than 15 inches of Hg will result in reduced genomic DNA yield and purity.

Following the second wash, it is critical to dry the Binding Plate for at least 6–10 additional minutes. Ethanol contamination in the DNA eluate can inhibit downstream applications such as PCR.

(a) Australian Pat. No. 730718, Singapore Pat. No. 64532 and Korean Pat. No. 486402 have been issued to Promega Corporation for an improved filtration system and method. Other patents are 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.