Automated Wizard® SV 96 PCR Clean-Up System

Automated Protocol #EP008

DESCRIPTION OF THE AUTOMATED METHODS WITH PRODUCTS A9340 and A9341
Please visit the web site to verify that you are using the most current version of this Automated Protocol.

1. Description

This document describes automation of the Wizard® SV 96 PCR Clean-Up System. Specific instructions are provided for the Beckman Biomek® 2000 and Biomek® FX and Eppendorf ep*Motion*® 5075 VAC automated liquid handling workstations. Validated methods for these liquid handling workstations are available at: **www.promega.com/automethods/**

General automation guidelines are provided for adaptation to other liquid handling platforms. For troubleshooting chemistry issues please refer to the *Wizard® SV 96 PCR Clean-Up System Technical Bulletin* #TB311.

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

2. Product Components

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

Each system contains sufficient reagents for 96 isolations. Includes:

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

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

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

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

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

Each system contains sufficient reagents for 8 × 96 isolations. Includes:

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

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

3. Before You Begin

Materials to Be Supplied by the User

- 80% ethanol **or** 95% ethanol (70ml/plate). Use 95% ethanol for purification of amplification products <500 bases.
- 96-well Greiner U-bottom plate (Promega Cat.# A9161)

3.A. Preparation of Solutions

Note: For purification of small amplification products (<500 bases), increase ethanol wash concentration to 95% for improved recovery.

Prepare a solution of 80% ethanol just before performing the automated clean-up procedure. An alcohol concentration lower than 80% will result in decreased sample recovery. For purification of small amplification products (<500 bases), increase the ethanol wash concentration to 95% for improved recovery.

3.B. Sample Preparation

Ensure that all sample is at the bottom of the 96-well PCR plate. If not, briefly spin the plate in a centrifuge to move all samples to the bottom of the wells.

4. Automated Processing Requirements for the Biomek® 2000 Workstation

4.A. Instrumentation Requirements for the Biomek® 2000

The following is a list of Beckman Coulter parts that are required for automation of the SV 96 PCR Clean-Up System on a Beckman Biomek® 2000 liquid handling workstation.

Description	Beckman 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
Gripper Tool System for Biomek® 2000	609001
Worksurface Spill Tray	609077
MP200 Pipetting Tool	609025
Tip Rack Holder (2 required for single plate run)	609121
Gray Labware Holder (2)	609120
Collar Holders	609736
Vacuum Valve Unit	609005
Vacuum Filtration Manifold Base	609670
36mm Vacuum Collar	609597
Elution Spacer	390792
Vacuum Regulator	609674
Tubing Kit, Filtration System	609676
Tubing Kit, Wash Unit	609687
Plastic Bottle, 4L	975796
Cap	975797
Reservoir Holder	372795
Quarter Single Reservoirs (2)	372790
Half Reservoir	372786

4.B. Labware Requirements for the Biomek® 2000

Labware Requirements	Source
96-well Elution Plate	provided in SV96 PCR Clean-Up System
SV 96 Binding Plate	provided in SV96 PCR Clean-Up System
96-well PCR plate	provided by user
96-well Greiner U-bottom plate	Promega Cat.# A9161 (4/pack)

Note: Only the base model of the Biomek® 2000 deck is used. The left and right side modules are not required.

4.C. Biomek® 2000 Deck Setup

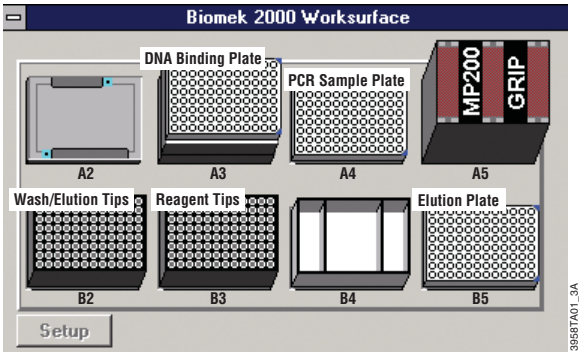


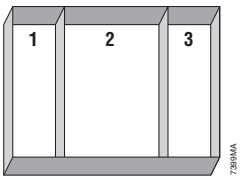
Figure 1. Biomek® 2000 initial deck configuration.

Deck Position(s)	Equipment
A2	collar holder
A3	vacuum filtration manifold base, elution spacer, 36mm collar, SV 96 Binding Plate
A4	labware holder, 96-well PCR sample plate sitting on a 96-well Greiner U-bottom plate.
A5	tool rack containing MP200, gripper tools
B2	tip rack holder, P250 tips
B3	tip rack holder, P250 tips
B4	labware holder, reservoir holder, two quarter single reservoirs and a half reservoir labware holder
B5	96-well Elution Plate

Reagent Dispense Volumes for the Biomek® 2000

Prior to beginning the run, dispense the following reagents as shown at position B4 on the deck of the Biomek® 2000.

Deck Position B4.



1. 15ml nuclease-free water
2. 70ml 80% (or 95%) ethanol (see Section 3.A)
3. 15ml Membrane Binding Solution

4.D. Biomek® 2000 Specific Pre-Run Recommendations

Before running, the method must be imported into the BioWorks™ Software. To import the method, please follow the instructions provided in the document: *Importing Biomek® 2000 Programs* (www.promega.com/automethods/beckman/biomek2000/default.asp). The Biomek® 2000 method is available at: **www.promega.com/automethods/**

5. Automated Processing Requirements for the Biomek® FX

5.A. Instrumentation Requirements for the Biomek® FX

The following is a list of Beckman Coulter parts that are required for automation of the SV 96 PCR Clean-Up System on a Beckman Biomek® FX liquid handling workstation.

Description	Beckman Part Number
Biomek® FX workstation	contact Beckman
Biomek® FX Software Version 2.1 or higher	contact Beckman
96-channel POD	contact Beckman
labware positions/POD (minimum of 10 required)	contact Beckman
Tip loader	contact Beckman
SPE ALP	contact Beckman
Holder ALP (for SPE)	contact Beckman
Vacuum Valve Unit	609005
Vacuum Filtration Manifold Base	609670
Elution Spacer	390792
36mm Vacuum Collar	609597
Tubing Kit, Filtration System	609676
Plastic Bottle, 4L	975796
Cap	975797

5.B. Labware Requirements for the Biomek® FX

Labware Requirements	
Pyramid-bottom Reservoir Plates (2)	Innovative Microplate Part# S30014
96-well Greiner U-bottom plates (3)	Promega Cat.# A9161(4/pack)
96-well Elution Plate	provided in SV 96 PCR Clean-Up System
non-skirted 96-well PCR plate	provided by user
Plate Stand	Promega Cat.# V8261
PCR Clamp 96	Promega Cat.# V8251
SV 96 Binding Plate	provided in SV 96 PCR Clean-Up System

5.C. Biomek® FX Deck Setup

Figure 2 shows an example of a deck layout for set-up of the SV 96 PCR Clean-Up System on the Biomek® FX. Your specific deck layout may be different depending on your Biomek® FX configuration.

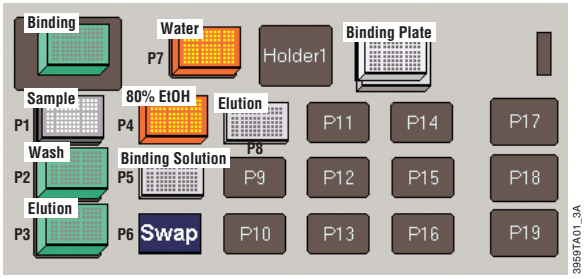


Figure 2. Biomek® FX initial deck configuration.

ALP Name	Equipment
Tip Loader	200µl non-ART® Biomek® FX Tips
P1	A non-skirted 96-well PCR plate in a Plate Clamp sitting on a Plate Stand.
P2	200µl non-ART® Biomek® FX Tips
P3	200µl non-ART® Biomek® FX Tips
P4	Pyramid bottom reservoir plate containing Xml 80% (or 95%) ethanol (see Section 3.A)
P5	Greiner 96-well U-bottom plate containing Xµl Membrane Binding Solution per well
P6	Swap spot
P7	Pyramid bottom reservoir plate containing Xml nuclease-free water.
P8	96-well Elution Plate
SPE ALP	Vacuum filtration manifold base, elution spacer, 36mm collar, SV 96 Binding Plate

Note: A minimum of 8 passive ALPs are required.

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

The Biomek® FX automated platform allows users the flexibility to configure the robot's deck according to need. Because of this flexibility, it is likely that the deck used for writing a Biomek® FX method will 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).

Prior to the first run of the SV 96 PCR Clean-Up method on the Beckman Biomek® FX, check all Gripper moves to ensure that the vacuum manifold disassembly and reassembly for elution is correct. We have found that Gripper moves for vacuum manifold disassembly and reassembly differ for each Biomek® FX instrument. Failure to do this Gripper test evaluation may result in vacuum manifold disassembly and reassembly failing and may even result in a Gripper crash. To check Gripper moves, please follow the instructions provided in the document: *Evaluation of Biomek® FX SV 96 Method Gripper Moves* (www.promega.com/automethods/beckman/biomekfx/default.asp).

Failure to perform Gripper test evaluation may result in vacuum manifold disassembly and reassembly failing and even a Gripper crash.

Evaluation of Biomek® FX SV 96 Purification Method Gripper Moves requires the Beckman Biomek® FX Grip Test method. Please inquire for this method.

6. Automated Processing Requirements for the ep*Motion*® 5075 VAC

6.A. Instrumentation Requirements for the ep*Motion*® 5075 VAC

The following is a list of Eppendorf parts that are required for automation of the SV 96 PCR Clean-Up System on an Eppendorf ep*Motion*® 5075 VAC liquid handling workstation.

Part Description	Eppendorf Part Number
ep*Motion*® 5075 VAC workstation, Gripper and Waste Tub	5075 000.016
TM 1000-8, 8-channel dispensing tool	5280 000.258
TM 300-8, 8-channel dispensing tool	5280 000.231
Reservoir Rack	5075 754.002
85mm Height adapter	5075 751.003
Vac Frame Holder	5075 752.000
Vac Frame 2	960002261

6.B. Labware Requirements for the ep*Motion*® 5075 VAC

Part Description	Ordering Information
100ml ep*Motion*® Reservoirs (3)	0030 126.513 (Eppendorf)
1,000µl ep*Motion*® Filter Tips (1)	0030 003.993 (Eppendorf)
300µl ep*Motion*® Filter Tips (1)	0030 003.977 (Eppendorf)
96-well Elution Plate	provided in SV 96 PCR Clean-Up System
SV 96 Binding Plate	provided in SV 96 PCR Clean-Up System
96-well Skirted PCR plate	provided by user
96-well Greiner U-bottom plate	Promega Cat.# A9161 (4/pack)

6.C. ep*Motion*® 5075 VAC Deck Setup

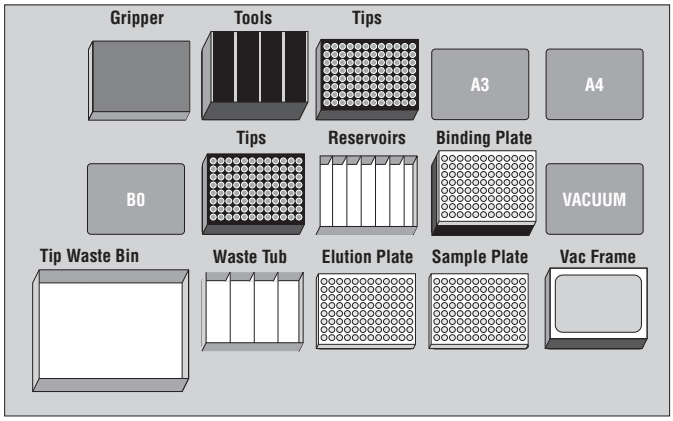


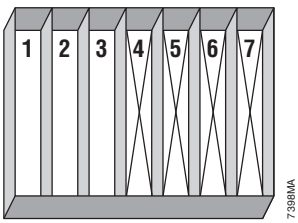
Figure 3. ep*Motion*® 5075 VAC initial deck configuration.

Deck Position	Equipment
T0	Gripper
T1	TM1000-8, 8-channel dispensing tool
T2	TM 300-8, 8-channel dispensing tool
T3	EMPTY
T4	EMPTY
A2	1000µl ep*Motion*® Filtered Tips
A3	EMPTY
A4	EMPTY
B0	EMPTY
B1	300µl ep*Motion*® Filtered Tips
B2	Reservoir Rack with 3 × 100ml Reagent Reservoirs
B3	Binding Plate on top of 85mm Height Spacer
Vacuum	EMPTY
C1	Waste Tub with quarter wall separators
C2	96-well Elution Plate
C3	96-well PCR Plate containing samples
C4	Vac Frame 2 on top of Vac Frame Holder

Reagent Dispense Volumes for the ep*Motion*® 5075 VAC

Prior to beginning the run, dispense the following reagents as shown at position B2 on the deck of the ep*Motion*® 5075 VAC workstation.

Deck Position B2. Place 100ml Reservoirs in rack at sites 1–3.



1. 15ml Membrane Binding Solution
2. 70ml 80% (or 95%) ethanol (see Section 3.A)
3. 15ml Nuclease-Free Water
4. EMPTY
5. EMPTY
6. EMPTY
7. EMPTY

7. Description of Automated SV 96 PCR Clean-Up

This overview describes the general liquid handling steps required for automated SV 96 PCR Clean-Up. The procedure can be adapted for performance on a variety of automated liquid handling robots. Additional information for adaptation to liquid handling robots other than those described here is provided in Section 8.

1. **Preparation of samples for binding to SV 96 Binding Plate**.
   An equal volume of Membrane Binding Solution is transferred to each sample in a 96-well PCR plate and mixed using pipet tips.
2. **Transfer samples to SV 96 Binding Plate**.
   The sample (with Membrane Binding Solution added) is transferred to the SV 96 Binding Plate that is sitting on top of the vacuum manifold apparatus.
3. **Bind nucleic acids to SV 96 Binding Plate**.
   Once all the sample has been transferred to the SV 96 Binding Plate, the vacuum is applied and sample is drawn through the Binding Plate for 30 seconds. During this step, nucleic acids bind to the SV 96 Binding Plate.
4. **Wash #1**.
   Two hundred microliters of 80% (or 95%, see Section 3.A) ethanol is dispensed to each well of the SV 96 Binding Plate. After a one-minute pause, the vacuum is applied for 30 seconds. The wash solution is pulled through the SV 96 Binding Plate.
5. **Washes #2 & #3**.
   Step 4 is repeated for a total of three washes of the SV 96 Binding Plate.
6. **Drying/removal of residual alcohol**.
   The vacuum remains on for four more minutes to remove any residual ethanol from the SV 96 Binding Plate.
7. **Preparation for elution**.
   After the final vacuum step there is a one-minute pause to allow for complete vacuum ventilation before disassembly and reassembly for the final elution step. A gripper tool disassembles the vacuum manifold stack by removing the SV 96 Binding Plate and vacuum collar from the vacuum manifold to holding position. The gripper then moves a 96-well Elution Plate into the vacuum manifold. The gripper then reassembles the vacuum manifold stack by moving the SV 96 Binding Plate and vacuum collar back onto the vacuum manifold over the top of the Elution Plate (Figure 3).
8. **Elution of purified amplification products**.
   One hundred microliters of Nuclease-Free Water is transferred from the reservoir to each well of the SV 96 Binding Plate. After a one-minute pause, the vacuum is applied. Nuclease-Free Water is pulled through the SV 96 Binding Plate, eluting the amplification products into the 96-well Elution Plate.
9. **Method ends**.
   Purified amplification products are eluted into the 96-well Elution Plate sitting in the vacuum manifold. Dispose of the SV 96 Binding Plate after use.

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Figure 4. Vacuum manifold disassembly, placement of Elution Plate and reassembly of vacuum manifold for elution of purified amplification products on the Beckman Biomek® 2000.

8. General Guidelines for Adaptation to Alternative Robotic Platforms

This method uses vacuum filtration for binding, washing and elution of samples. Make sure that the vacuum pump you are using is set to pull a vacuum of 15–20 inches Hg to ensure that sufficient pressure is applied. Vacuum pressure less than 15 inches of Hg may reduce recovery and may cause column clogging. Vacuum pressure >20 inches may result in spraying.

An 80% ethanol wash solution is recommended for optimal removal of primers and nucleotides from amplification products larger than 500 bases. For optimal recovery of small amplification products (<500 bases) we recommend increasing the ethanol concentration to 95%.

Pause steps built into the purification procedure improve binding, wash and elution. Removal of these pauses may decrease the purity and recovery of amplification products.

Following Wash #3, drying of the Wizard® SV 96 Binding Plate for at least 4 additional minutes is critical to remove residual ethanol. This drying step may need to be extended for more than 4 minutes to make sure that all residual ethanol is removed. Ethanol contamination in the purified eluate may cause inhibition of downstream reactions.

The recommended elution volume for the SV 96 PCR Clean-Up System is 100µl. This results in approximately 60–70µl of eluate. Decreases in elution volume may increase the concentration of eluted product but may also result in a decrease in recovery.