AUTOMATED PROTOCOL

Automated SV 96 Total RNA Isolation System

Instructions for use of the Automated Methods with Products
Z3505 and Z3500

Revised 9/16
EP003

Automated SV 96 Total RNA Isolation System

DESCRIPTION OF THE AUTOMATED METHODS WITH PRODUCTS Z3500 AND Z3505.
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 SV 96 Total RNA Isolation System. Specific instructions are provided for the Beckman Coulter Biomek® 2000, Biomek® 3000 and Biomek® FX and the Eppendorf ep*Motion*® 5075 automated liquid-handling workstations. Information on obtaining validated methods for these workstations is 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 *SV 96 Total RNA Isolation System Technical Bulletin* #TB294.

2. Product Components

Product	Size	Cat.#
SV 96 Total RNA Isolation System	1 × 96	Z3500

Each system contains sufficient reagents for 96 isolations. Includes:

- 50ml RNA Lysis Buffer (RLA)
- 1 × 0.9ml β-mercaptoethanol (97.4%)
- 1 vial DNase I (lyophilized)
- 750µl $MnCl_2$, 0.09M
- 2.5ml Yellow Core Buffer
- 13.25ml DNase Stop Solution (DSA)
- 58.8ml RNA Wash Solution (RWA)
- 13ml Nuclease-Free Water
- 1 Binding Plate
- 1 Elution Plate* (wrapped)
- 3 Plate Sealers

*A second, unwrapped plate is used in the packaging to protect the nibs on the Binding Plate. Do not use this unwrapped plate for elution.

Product	Size	Cat.#
SV 96 Total RNA Isolation System	5 × 96	Z3505

Each system contains sufficient reagents for 5 × 96 isolations. Includes:

- 2 × 50ml RNA Lysis Buffer (RLA)
- 2 × 0.9ml β-mercaptoethanol (97.4%)
- 5 vials DNase I (lyophilized)
- 5 × 750µl $MnCl_2$, 0.09M
- 5 × 2.5ml Yellow Core Buffer
- 5 × 13.25ml DNase Stop Solution (DSA)
- 5 × 58.8ml RNA Wash Solution (RWA)
- 5 × 13ml Nuclease-Free Water
- 5 Binding Plates
- 5 Elution Plates* (wrapped)
- 6 Plate Sealers

*A sixth, unwrapped plate is used in the packaging to protect the nibs on the Binding Plates. Do not use this unwrapped plate for elution.

3. Materials to Be Supplied by the User

- ethanol, 95%, RNase-free (120ml per 96-well plate)
- 10X phosphate-buffered saline (PBS), sterile (for cultured cells)

For Biomek platforms:

- vacuum pump capable of 15–20 inches of Hg (e.g., Fisher Cat.# 01-092-29)
- vacuum trap for waste collection
- vacuum tubing (e.g., neoprene tubing, Fisher Cat.# 14-171B)
- **for Biomek® FX only**: Pyramid Bottom Reservoir Plates (2; Cat.# V6801)

4. Before You Begin

4.A. Preparation of Solutions

Prior to beginning the procedure with a new SV 96 Total RNA Isolation System, dilute the provided solutions as follows:

RNA Lysis Buffer: Add 0.5ml of β-mercaptoethanol (BME) to 50ml of RNA Lysis Buffer (RLA). After adding BME, mark on the bottle that this step has been performed. Store the RNA Lysis Buffer with BME at 4°C.

DNase I: Add 312.5µl of Nuclease-Free Water (supplied) to the lyophilized DNase I. Gently mix by swirling. Do not vortex. One vial is sufficient for one 96-well plate. If processing less than a whole plate, we recommend dividing the rehydrated DNase into working aliquots using sterile, RNase-free microcentrifuge tubes. Each RNA purification requires 2.5µl of rehydrated DNase I. Store the rehydrated DNase I at –20°C.

RNA Wash Solution: Add 100ml of 95% ethanol to the bottle containing 58.8ml of concentrated RNA Wash Solution (RWA). After adding ethanol, mark on the bottle that this step has been performed. The RNA Wash Solution is stable at 22–24°C when tightly capped.

DNase Stop Solution: Add 20ml of 95% ethanol to the bottle containing 13.25ml of concentrated DNase Stop Solution (DSA). After adding ethanol, mark on the bottle that this step has been performed. The DNase Stop Solution is stable at 22–25°C when tightly capped.

4.B. Sample Preparation Before Automated Processing

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

Note: Throughout this document, RNA Lysis Solution (RLA), RNA Wash Solution (RWA) and DNase Stop Solution (DSA) refer to the solutions supplied with the SV RNA Isolation System. Once prepared as described in Section 4.A, these solutions are referred to as RNA Lysis Buffer, RNA Wash Solution and DNase Stop Solution.

5. Description of Automated Processing Requirements

5.A. Biomek® 2000 Workstation Requirements

The following is a list of Beckman Coulter parts and their corresponding part numbers that are required to automate the SV 96 Total RNA Isolation System on a Biomek® 2000 workstation.

Instrument Requirements for the Biomek® 2000 Workstation

Part Description	Beckman Coulter Part Number
Biomek® 2000 Workstation, 50/60Hz, 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
Wash 8 Tool	609027
Wash Unit with 6-Port Valve	609056
MP200 Pipetting Tool	609025
Tip Rack Hoder (2 for single plate run)	609121
Gray Labware Holder (3)	609120
Collar Holders (1)	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 (1)	372795
Quarter Single Reservoirs (2)	372790
Quarter Vertical Reservoir (1)	372788

Labware Requirements for the Biomek® 2000 Workstation

Part Description	Quantity*	Ordering Information
96-Well Elution Plate	1	Provided in SV 96 Total RNA Isolation System Kit
Binding Plate	1	Provided in SV 96 Total RNA Isolation System Kit
96-well cell plate	1	Clear-well, 96-well flat-bottom tissue culture plate provided by the user

*per 96-well plate processed

Initial Deck Configuration for the Biomek® 2000 Workstation

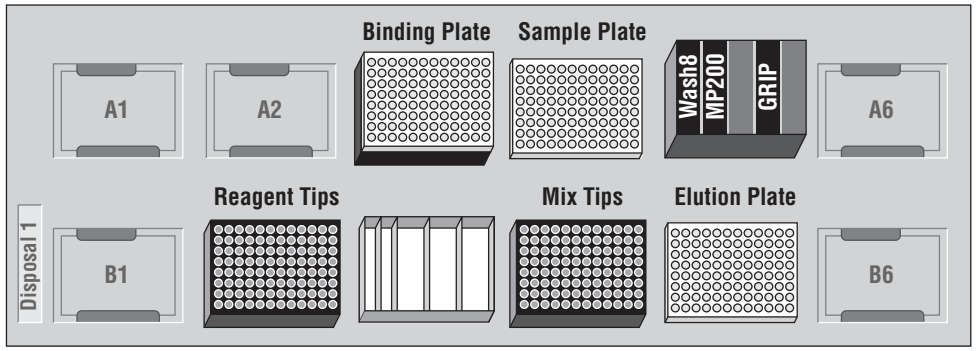


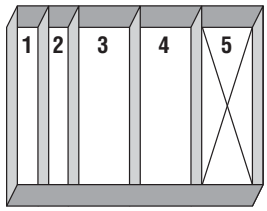
Figure 1. Biomek® 2000 Workstation Initial Deck Configuration.

Position A2	Collar Holder
Position A3	Vacuum filtration manifold base, elution spacer, 36mm collar, Binding Plate
Position A4	Labware holder, 96-well, flat-bottom sample plate
Position A5	Tool rack containing Wash 8, MP200 and Gripper tools
Position B2	Tip rack holder, P250 tips
Position B3	Labware holder, reservoir holder, two quarter single reservoirs and a quarter vertical reservoir
Position B4	Tip rack holder, P250 tips
Position B5	Labware holder, 96-well, flat-bottom elution plate

Reagent Dispense Volumes for the Biomek® 2000 Workstation

Prior to beginning the run, the following SV 96 System reagents need to be dispensed appropriately on the deck of the Biomek® 2000 workstation according to the initial deck configuration.

Position B3



1. 12ml Nuclease-Free Water
2. 3.125ml DNase Solution (312.5µl DNase, 312.5µl $MnCl_2$, 2.5ml Yellow Core Buffer)
3. 22ml DNase Stop Solution (ethanol added)
4. 12ml RNA Lysis Buffer (BME added)
5. EMPTY

Valve 1 of the Biomek® Wash Unit should be connected to the RNA Wash Solution (Ethanol Added).

Pre-Run Biomek® 2000 Workstation Specific Recommendations

Before running the method, import the method into the BioWorks™ Software. Please follow the instructions for Importing Biomek® 2000 Methods: **www.promega.com/automethods/**

5.B. Biomek® 3000 Workstation Requirements

The following is a list of Beckman Coulter parts and their corresponding part numbers that are required to automate the SV 96 Total RNA Isolation System on a Biomek® 3000 workstation.

Instrument Requirements for the Biomek® 3000 Workstation

Part Description	Quantity	Beckman Coulter Part Number
Biomek® 3000 Workstation, 50/60Hz, 100–120V	1	986120
Biomek® 3.3 Software	1	719349
PC with Windows® XP OS	1	987820
LCD Display, Flat, 17”	1	A18659
Gripper Tool Kit	1	A09053
8-Channel Wash Tool	1	987370
Wash Unit with Automatic Six-Port Valve	1	609056
Left-Side Module	1	987264
MP200 Pipette Tool	1	986146
Black Tip Rack Holder	2	391910
Gray Labware Holder	3	609120
96-Filtration System	1	A15925
5.0 L Reservoir Bottle	1	148029
Modular Reservoir Frame	1	372795
Quarter Vertical Reservoir	1	372788
Quarter Single Reservoir	2	372790

Labware Requirements for the Beckman Biomek® 3000 Workstation

Part Description	Quantity*	Ordering Information
96-well Elution Plate	1	Provided in SV 96 Total RNA Isolation System
Binding Plate	1	Provided in SV 96 Total RNA Isolation System
96-well cell plate	1	Clear-well, 96-well flat-bottom tissue culture plate provided by user

*per 96-well plate processed

Initial Deck Configuration for the Biomek® 3000 Workstation

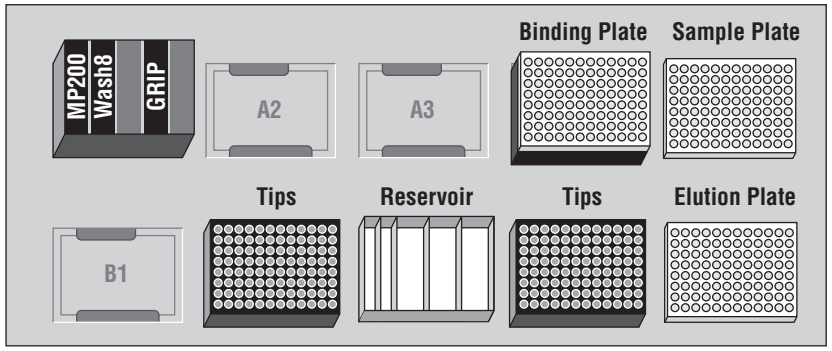


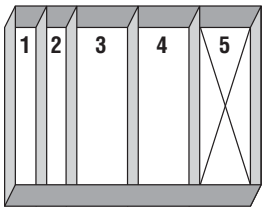
Figure 2. Biomek® 3000 Workstation Initial Deck Configuration.

Position A1	Tool rack containing MP200, Wash 8, Gripper tools
Position A2	Empty
Position A3	Empty
Position A4	Vacuum filtration manifold base, elution spacer, 36mm collar, Binding Plate
Position A5	Labware holder, 96-well, flat-bottom sample plate
Position B1	Empty
Position B2	Tip rack holder, P250 tips
Position B3	Labware holder, reservoir holder, two quarter single reservoirs, 1 quarter vertical reservoir
Position B4	Tip rack holder, P250 tips
Position B5	Labware holder, 96-well, flat-bottom elution plate

Reagent Dispense Volumes for the Biomek® 3000 Workstation

Prior to beginning the run, the following SV 96 System reagents need to be dispensed appropriately on the deck of the Biomek® 3000 workstation according to the initial deck configuration.

Position B3



1. 12ml Nuclease-Free Water
2. 3.125ml DNase Solution (312.5µl DNase, 312.5µl $MnCl_2$, 2.5ml Yellow Core Buffer)
3. 22ml DNase Stop Solution (ethanol added)
4. 12ml RNA Lysis Buffer (BME added)
5. EMPTY

Valve 1 of the Biomek® Wash Unit should be connected to the RNA Wash Solution (Ethanol Added).

Pre-Run Biomek® 3000 Workstation Specific Recommendations

Before running the method, import the method into the Biomek® Software. Please follow the instructions for Importing Biomek® 3000 Methods: **www.promega.com/automethods/**

Prior to the first run of the SV 96 Total RNA Purification method on the Biomek® 3000, check all Gripper moves to ensure that the vacuum manifold disassembly and reassembly for elution is correct. Proper configuration of the Gripper moves is essential to ensure the success of SV 96 methods on the Biomek® 3000. Not performing the Gripper test evaluation may result in failure of vacuum manifold disassembly and reassembly and may damage your instrument.

5.C. Biomek® FX Workstation Requirements

The following is a list of Beckman Coulter parts and their corresponding part numbers that are required to automate the SV 96 Total RNA Isolation System on a Biomek® FX workstation.

Instrument Requirements for the Biomek® FX Workstation

Part Description	Quantity	Beckman Coulter Part Number
Minimum: Biomek® FX Software version 2.1		Contact Beckman Coulter
96-channel POD	1	Contact Beckman Coulter
Minimum number of Labware Positions by 1 POD	10	Contact Beckman Coulter
Tip Loader	1	Contact Beckman Coulter
SPE ALP	1	Contact Beckman Coulter
Holder ALP (for SPE)	1	Contact Beckman Coulter
Vacuum Valve Unit	1	609005
Vacuum Filtration Manifold Base	1	609670
Elution Spacer	1	390792
36mm Vacuum Collar	1	609597
Tubing Kit, Filtration System	1	609676
Plastic Bottle, 4L	1	975796
Cap	1	975797

Labware Requirements for the Beckman Biomek® FX Workstation

Part Description	Quantity*	Ordering Information
Pyramid Bottom Reservoir Plates	2	Promega Cat.# V6801
96-well U-bottom Plates	3	Promega Cat.# A9161 (4 pk)
96-well Elution Plate	1	Provided in SV 96 Total RNA Isolation System
96-well cell plate	1	Clear-well, 96-well flat-bottom tissue culture plate provided by user
Binding Plate	1	Provided in SV 96 Total RNA Isolation System

*per 96-well plate processed

Initial Deck Configuration for the Biomek® FX Workstation

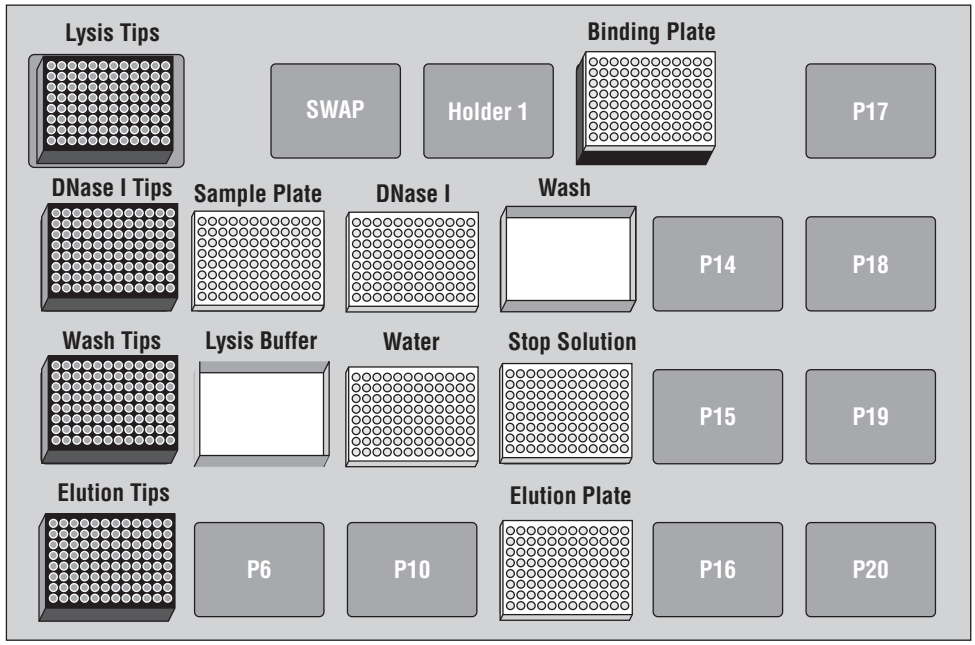


Figure 3. Biomek® FX Workstation Initial Deck Configuration.

ALP Name	Part Siting on ALP
Tip Loader	200µl non-ART Biomek® FX tips
P1	200µl ART Biomek® FX tips
P2	200µl non-ART Biomek® FX tips
P3	200µl ART Biomek® FX tips
P4	96-well, flat-bottom sample plate
P5	Pyramid bottom reservoir plate containing 40ml RNA Lysis Buffer (BME added)
P7	Swap spot
P8	Greiner 96-well, round-bottom plate containing 31µl of DNase Solution per well
P9	Greiner 96-well, round-bottom plate containing 125µl nuclease-free water per well
P11	Pyramid bottom reservoir plate containing 130ml RNA Wash Solution (ethanol added)
P12	Greiner 96-well, round-bottom plate containing 225µl DNase Stop Solution per well
P13	96-well, flat-bottom elution plate
SPE ALP	SPE ALP: Vacuum filtration manifold base, elution spacer, 36mm collar, Binding Plate

5.C. Biomek® FX Workstation Requirements (continued)

Pre-Run Biomek® FX Specific 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, the deck used for writing a Biomek® FX method is likely to 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 provided: Biomek® FX Deck Mapping (**www.promega.com/automethods/**)

Prior to the first run of the SV 96 Total RNA Purification method on the Biomek® FX, check all Gripper moves to ensure that the vacuum manifold disassembly and reassembly for elution is correct. Proper configuration of the Gripper moves is essential to ensure the success of SV 96 methods on the Biomek® FX. Not performing the Gripper test evaluation may result in failure of vacuum manifold disassembly and reassembly and may damage your Biomek® FX instrument.

Follow the instructions provided: Evaluation of Biomek® FX SV 96 Purification Method Gripper Moves (**www.promega.com/automethods/**)

Evaluation of Biomek® FX SV 96 Purification Method Gripper Moves requires the Biomek® FX method: "BFXSV96griptest". This method can be obtained by contacting Promega.

5.D. ep*Motion*® 5075 Workstation Requirements

The following is a list of Eppendorf parts and their corresponding part numbers that are required to automate the SV 96 Total RNA Isolation System on an ep*Motion*® 5075 workstation.

Instrument Requirements for the ep*Motion*® 5075 Workstation

Labware Requirements for the Eppendorf ep*Motion*® 5075 Workstation

Part Description	Quantity	Eppendorf Part Number
ep*Motion*® 5075 VAC workstation, Gripper and Waste Tub	1	5075 000.016
TM 300-8, 8-channel dispensing tool	1	5280 000-231
TM 1000-8, 8-channel dispensing tool	1	5280 000-258
Reservoir Rack (for 30ml and 100ml reagent reservoirs)	1	5075 754.002
85mm Height adapter	1	5075 751.003
55mm Height adapter	1	5075 752.000
Vac Frame 2	1	Contact Eppendorf

Part Description	Quantity*	Eppendorf Part Number
30ml ep*Motion*® Reservoir	1	0030 126.505
100ml ep*Motion*® Reservoir	1	0030 126.513
300µl ep*Motion*® Filter Tips, volume range 20–300µl	1	0030 003.977
1,000µl ep*Motion*® Filter Tips, volume range 40–1,000µl	1	0030 003.993
96-well Elution Plate	1	Provided in SV 96 Total RNA Isolation System Kit
Binding Plate	1	Provided in SV 96 Total RNA Isolation System Kit
96-well cell plate	1	Clear-well, 96-well flat-bottom tissue culture plate provided by the user

*per 96-well plate processed

Initial Deck Configuration for the ep*Motion*® 5075 Workstation

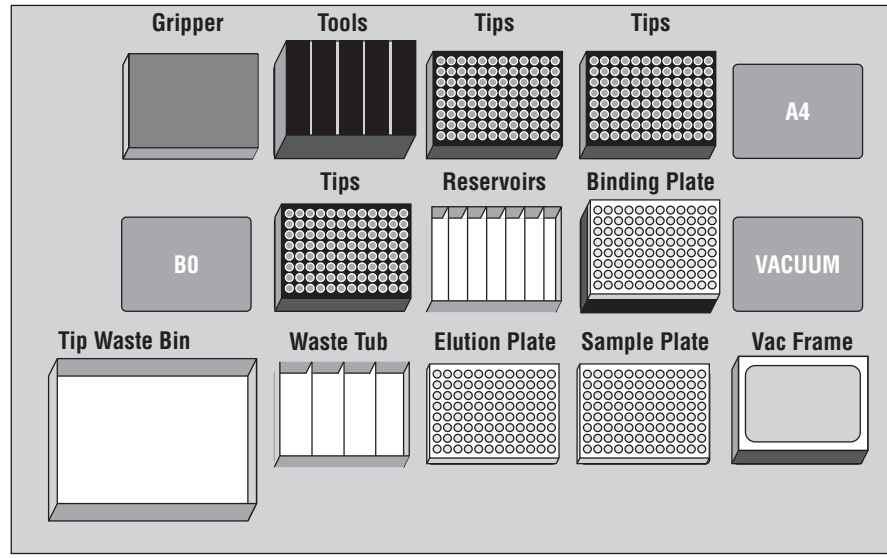


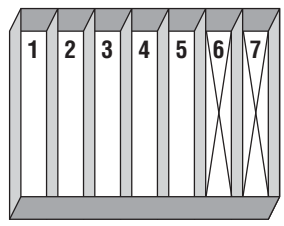
Figure 4. ep*Motion*® 5075 Workstation Initial Deck Configuration.

Position A2	1000µl epTIPS Motion Filtered Tips
Position A3	300µl epTIPS Motion Filtered Tips
Position A4	EMPTY
Position B0	EMPTY
Position B1	300µl epTIPS Motion Filtered Tips
Position B2	Reservoir Rack with 5 Reagent Reservoirs
Position B3	Binding Plate atop 85mm Height Spacer
Position Vacuum	EMPTY
Position C1	Waste Tub with quarter wall separators
Position C2	96-well Elution Plate
Position C3	96-well Flat-bottom Tissue Culture Sample Plate
Position C4	Vac Frame 2 on 35mm Height Spacer

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

Prior to beginning the run, the following SV 96 System reagents need to be dispensed appropriately on the deck of the ep*Motion*® 5075 workstation according to the initial deck configuration.

Position B2



1. 100ml Reservoir: 12ml RNA Lysis Buffer (BME added)
2. 30ml Reservoir: 3.125ml DNase Solution (312.5µl DNase, 312.5µl $MnCl_2$, 2.5ml Yellow Core Buffer)
3. 100ml Reservoir: 22ml DNase Stop Solution (ethanol added)
4. 100ml Reservoir: 100ml RNA Wash Solution (ethanol added)
5. 100ml Reservoir: 12ml Nuclease-Free Water
6. EMPTY
7. EMPTY

6. Description of Automated SV 96 Total RNA Isolation

This overview describes the general liquid handling steps required for automated SV 96 Total RNA Isolation 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 Section7. *General Guidelines for Adaptation to Alternative Robotic Platforms*.

1. **Cell Lysis.** One hundred microliters of RNA Lysis Buffer is transferred from a reservoir to the 96-well, flat-bottom sample plate containing cells alone. The contents are mixed with pipet tips to completely lyse cells.
2. **Transfer Cell Lysates.** The cell lysate contained in the 96-well, flat-bottom sample plate is transferred to the SV 96 Binding Plate sitting on top of the vacuum manifold apparatus.
3. **Bind Total RNA to Binding Plate.** Once all the cell lysate has been transferred to the Binding Plate, the vacuum is applied, and cell lysate is pulled through the Binding Plate by vacuum for one minute. During this vacuum, total RNA binds to the Binding Plate.
4. **Wash #1 Binding Plate.** Five hundred microliters of RNA Wash Solution is dispensed to each well of the Binding Plate. Vacuum is applied, and the Wash Solution is drawn through the Binding Plate by vacuum for one minute.
5. **DNase Treat Samples.** Twenty five microliters of prepared DNase Solution is transferred from the reservoir to each well of the Binding Plate. The robot pauses for 10 minutes to allow for DNase incubation. DNase incubation is typically 10 minutes but can be lengthened to 20 minutes. We do not recommend greater than a 20-minute DNase incubation.
6. **DNase Stop Solution.** The DNase is inactivated by adding 200µl of DNase Stop Solution from the reservoir to each well of the Binding Plate. Vacuum is applied and the DNase Stop Solution is drawn through the Binding Plate by vacuum for 30 seconds.
7. **Wash #2 Binding Plate.** Five hundred microliters of RNA Wash Solution is dispensed to each well of the Binding Plate. Vacuum is applied, and the RNA Wash Solution is pulled through the Binding Plate by vacuum for one minute.
8. **Drying/Removal of Residual Alcohol.** The vacuum remains on for three more minutes to remove any residual ethanol from the Binding Plate.
9. **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 Binding Plate and manifold collar from the vacuum manifold to holding position. The Gripper then moves the 96-well, flat-bottom elution plate into the vacuum manifold and reassembles the vacuum manifold stack by moving the Binding Plate and manifold collar back onto the vacuum manifold.

6. Description of Automated SV 96 Total RNA Isolation (continued)

Figure 5. Example of vacuum manifold disassembly, placement of the elution plate and reassembly of vacuum manifold for elution of purified total RNA on the Beckman Biomek® 2000. Panel A. Disassembly of vacuum manifold. **Panel B.** Placement of Elution Plate. **Panel C.** Reassembly of vacuum manifold.

10. **Elution of Purified Total RNA.** One hundred microliters of nuclease-free water is transferred from the reservoir to each well of the Binding Plate. Incubate at room temperature for 1 minute. Vacuum is applied, and the Nuclease-Free Water is pulled through the Binding Plate, eluting the total RNA into the 96-well Elution Plate.
11. **Method Ends.** Purified total RNA has been eluted into the 96-well, Elution Plate. Dispose of the Binding Plate after use.

7. General Guidelines for Adaptation to Alternative Robotic Platforms

Because the SV 96 Total RNA Isolation System is used to isolate RNA from tissue culture cells and tissue lysates, we recommend using aerosol-resistant tips for this method to decrease the chance of contaminating samples with RNases. If your robotic platform uses fixed tips, be sure that the tips are washed thoroughly between pipetting steps. Also, if system liquid is used to perform pipetting steps, be sure to limit the exposure of samples to system liquid (a potential source of RNase contamination) during all pipetting steps by increasing the volume of leading air gaps that are used for pipetting.

This method uses vacuum filtration of samples for binding, washing, and elution. Make sure that the vacuum pump you are using is set to pull a vacuum of 15–20 inches Hg to ensure that a sufficient vacuum pressure is being used. Vacuum pressure less than 15 inches of Hg will result in reduced purified total RNA yield and purity and may cause column clogging when processing tissue lysates.

DNase I Solution volumes provided in the SV 96 RNA Isolation Kit are limiting. Therefore, use exactly the recommended volumes described in this Automated Technical Bulletin. When performing the DNase I treatment of samples on the membrane of the Binding Plate, be sure to pause 10 minutes to degrade genomic DNA before adding the DNase Stop Solution.

Following Wash #2, drying the SV 96 Binding Plate for at least 3 additional minutes is critical to remove residual ethanol. This drying step may need to be extended for more than 3 minutes to make sure that all residual ethanol is removed. Ethanol contamination in the RNA eluate can inhibit downstream reactions such as RT-PCR.

The recommended elution volume for the SV 96 Total RNA Isolation System is 100µl resulting in approximately 60–70µl of eluted RNA. Decreases in elution volume will result in concomitant decreases in the volume of eluted material and RNA yield.

8. Summary of Changes

The following changes were made to the 9/16 revision of this document:

1. The amount and concentration of the β-Mercaptoethanol component was changed, Sections 2 and 4.A.