# Instructions For Use

# Biomek 4000

**RNAdvance Blood Application** 

Total RNA Isolation from PAXgene Preserved Blood

B39494AA October 2013



Beckman Coulter, Inc. 250 S. Kraemer Blvd. Brea, CA 92821 U.S.A.



#### **Biomek 4000 RNAdvance Blood Application Instructions for Use** PN B39494AA (October 2013)

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# **Revision History**

This document applies to the latest software listed and higher versions. When a subsequent software version changes the information in this document, a new issue will be released.

**AA**/**Initial Issue, October 2013** Biomek Software version 4.1 or higher **Revision History** 

# Safety Notice

Read all product manuals and consult with Beckman Coulter-trained personnel before attempting to operate instrument. Do not attempt to perform any procedure before carefully reading all instructions. Always follow product labeling and manufacturer's recommendations. If in doubt as to how to proceed in any situation, contact your Beckman Coulter Representative.

# Alerts for Warning, Caution, Important, and Note

#### 

WARNING indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.

### <u> 🕂</u> CAUTION

CAUTION indicates a potentially hazardous situation, which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

- **IMPORTANT** IMPORTANT is used for comments that add value to the step or procedure being performed. Following the advice in the Important adds benefit to the performance of a piece of equipment or to a process.
- **NOTE** NOTE is used to call attention to notable information that should be followed during installation, use, or servicing of this equipment.

Safety Notice Alerts for Warning, Caution, Important, and Note

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Beckman Coulter, Inc. Customer End User License Agreement

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# Introduction

# **Intended Use**

The product is not intended or validated for use in the diagnosis of disease or other conditions.

# **Overview**

This introductory section contains the following topics:

- How to Use This Manual
- About This Manual

# How to Use This Manual

Use this manual to configure, test and operate the RNAdvance Blood method. It contains information on:

- Instrument Requirements
- System Configuration Settings
- Method Operation

## **About This Manual**

**NOTE** Screens in this manual may differ slightly from the screens in your RNAdvance Blood method. The information in this *Instructions for Use* manual is organized as follows:

#### **Chapter 1, Instrument Requirements**

Contains deck configuration and general settings for operating the RNAdvance Blood method.

#### **Chapter 2, Method Operation**

Contains the settings and procedures for operating the RNAdvance Blood method. The manual also includes a List of Abbreviations, a Glossary, and an Index.

# Conventions

- Selections that appear on a screen are in **bold face**.
- Information that is to be typed (entered) is in **bold face and italics** font.
- The software path to a specific function or screen appears with the greater than ( > ) symbol between succeeding screen options, like this: Select **Control Panel > Network and Sharing**.
- Links to information in another part of the document for additional information are in blue. To access the linked information, select the blue text.



# **Instrument Requirements**

Biomek 4000 with software version 4.1 or higher. Contact your Beckman Coulter Representative for the Method CD for your platform, call 1-800-369-0333, or email *reagentsupport@beckman.com* if your sales contact information is unknown.

## **Deck Configuration**

See Figure 1.1 for the recommended deck layout.



Figure 1.1 Recommended Deck Layout

Table 1.1 Requirements for RNAdvance Blood Method

Туре	Qty	Description	Part number
Tools	1	Gripper Tool	987371
	1	MP200 Eight-Tip Pipette Tool	986146
	1	MP1000 Eight-Tip Pipette Tool	A91112

Туре	Qty	Description	Part number	
ALPs	1	Liquid Waste ALP	B21398	
	1	Disposal ALP	609751	
	5	Labware Holder	609120	
	4	Tip Rack Holder	391910	
	1	Static Peltier Device	A93938	
	1	Orbital Shaker	379448	
	1	Peltier Adaptor Plate (96 well round bottom)	A49568	
	1	Off Deck Tool Rack Kit (left)	B21395	
Magnet Plate	1	Super Magnet Plate	A32782	
Reservoirs   2   Reservoir Frame		372795		
2Half Reservoir33Quarter Reservoir3		372786		
		372790		
	1	IMReservoir96 (Fisher Scientific)	50-995-860	
	1	Quarter Reservoir (divided by length)	372788	
Consumables3Biomek Span-8 P1000 Tips, Pre-Sterile with Barrier		Biomek Span-8 P1000 Tips, Pre-Sterile with Barrier	B01124	
	1	Biomek AP96 P250 Tips, Pre-Sterile with Barrier	717253	
	1	96 Well PCR Plate <sup>a</sup>	AB-2800	
	1	RK Riplate Deepwell Plate (Worldwide Medical)	99181000	

 Table 1.1 Requirements for RNAdvance Blood Method

a. ABGene 2800 or equivalent.

1

# **System Configuration Settings**

## **Customer Default Settings**

The system configuration settings section contains additional selections. Adjust the following fields as needed.

#### To adjust user defaults and settings

- **1** Open the Biomek Software.
- **2** Open the RNAdvance Blood method by clicking **File > Open**. The method opens and the method outline displays. See Figure 1.2.

Figure 1.2 Configure Method Outline



**3** From the method outline click **User Defaults and Settings**. See Figure 1.2. The screen in Figure 1.3 displays.

#### Figure 1.3 RNAdvance Blood Customer Default Values

Prep Plate Information					
Blood volume	400	μL	(200-500)	(default: 400)	ſ
<b>Processing Information</b>					
Lysis					
Lysis volume	300	μL	<mark>(100-500)</mark>	(default: 300)	
Proteinase K Solution volume	40	μL	(20-100)	(default: 40)	
Lysis Shake speed	1200	RPM	(1000-1200)	(default: 1200)	
Lysis Shake time	3	min	<mark>(1-60)</mark>	(default: 3)	
Lysis Sample Temperature	55	°C	(20-100)	(default: 55)	
Lysis Incubation time	25	min	<b>(1-60)</b>	(default: 25)	
·····					
Elution	1	min		ac 1)	
Flution Settle time	2	min	(1-5)	(default: 2)	
Transfer • Yes O No	1-		(10)	(doldali. 2)	
Eluent Transfer volume	35	μL	(20-100)	(default: 35)	
- Reset to Default Values	,				
LIN	IS Settings				
-LIMS Path: C:\					-
Pal	tier Offsets				
				_	
— Lysis Peltier temperature offset			40	°C	
— DNase Peltier temperature offset			13	°C	
The Reset to Default Values button does not determined during installation and are meant to	ensure your sa	r tempera amples re	ature offsets. The each the desire	iese offsets are d temperature	
during method execution. The actual Peltier ter	nperature will b	e equal t	o the sum of th	e sample	
temperature plus die reduci temperature of					

1

Screen Element	Purpose	Do this
A Default Protocol Settings	These values can be adjusted for customer specified processes. These values will feed into the application interface as the default values.	Change values as needed based on your specific protocol.
B Reset to Default Values	Resets all values (except the Peltier Offset values), back to the factory default values.	Click the button to revert all values to the default values.
C LIMS Folder	File location to save Laboratory Information Management System (LIMS) data.	Enter the file path where you would like application data saved. For example: <i>c:\Program Files\LIMS</i>
D Peltier Offsets	During installation the Peltier Offset temperature values are determined based on the particular Peltier installed. Only change these values when recommended by Beckman Coulter.	Do not change.

Table 1.2 RNAdvance Blood Customer Default Value Descriptions

**NOTE** It is recommended that you save the new version of the method under a different name to preserve the original. This allows you to go back to the original as a starting point if needed.

**IMPORTANT** Changing default protocol settings may cause invalid method results. Beckman Coulter's warranty applies only for unchanged Customer Default Protocol Settings.

To restore the Beckman Coulter Agencourt Default settings, click the Reset to Default Values Button.

- **IMPORTANT** The Reset to Default Values button does not reset the Peltier temperature offsets. These offsets are determined during installation and are meant to ensure your samples reach the desired temperature during method execution. The actual Peltier temperature is equal to the sum of the set temperature, plus the offset temperature. The formula would be as follows:
  - Sample Temp + Peltier Temp Offset = Actual Peltier Temp
  - For example, to determine the actual temperature of the Peltier for Lysis:

If the Lysis Temperature is set to 55°C, and the Lysis Peltier Temperature Offset is set to 40°C, the actual temperature of the Peltier would be:

55°C + 40°C = **95**°**C** 

# Installation

Your Beckman Coulter Field Applications Scientist performs all Biomek and RNAdvance Blood Application installation and setup services. For service, see your Beckman Coulter Representative.

# **Operating the RNAdvance Blood Method**

**NOTE** For more information about Agencourt RNAdvance Blood and the Agencourt RNAdvance Blood protocol, see www.beckmancoulter.com. The automated method has been optimized for automation and may differ from the manual protocol.

# Starting the RNAdvance Blood Method and Establishing Default Protocol Settings

The RNAdvance Blood Method is shipped with a set of standard protocol values. But these values may need to be modified for your laboratory. These values populate the RNAdvance Blood method application.

#### To open and start the method

- **1** Open the Biomek software.
- **2** Open the RNAdvance Blood Project by clicking **Project** > **Open Project**.

- **3** Open the RNAdvance Blood method by clicking **File > Open**. Select **RNAdvanceMethod**.
  - **NOTE** Your method structure may look larger and may not have + and icons to expand and collapse the structure. To change the appearance of the method structure, click **Options** > **Preferences** and then click **View** in the menu bar on the left. Use this option to change the appearance of the method structure.

Figure 2.1 Method Outline



**4** From the method outline, click **User Defaults and Settings**. See Figure 2.1. The User Defaults and Settings screen displays.

NOTE You can skip steps 4 and 5 if already configured previously.

Figure 2.2 User Defaults and Settings, Top portion shown	
--	--

Customo		aluaa			1
Pren Plate Information	r Delault v	alues			
Blood volume	400	ul	(200-500)	(default: 400)	ľ
Processing Information	1		(/	()	
Lyeie					
Lysis volume	300	ul	(100-500)	(default: 300)	
Proteinase K Solution volume	40	ul.	(20-100)	(default: 40)	
Lysis Shako spood	1200		(1000 1200)	(default: 1200)	
Lysis Shake speed	2	min	(1000-1200)	(default: 2)	
Lysis Shake unle		•	(1-00)	(default: 5)	Ļ
Lysis Sample Temperature	05	6	(20-100)	(default: 55)	
Lysis incubation time	25	min	(1-60)	(default: 25)	
	440		(000 500)		
Bind 1 volume	410	μL	(200-500)	(default: 410)	
Bind 1 Tipmixing	15	time(s)	(1-20)	(default: 15)	
Bind 1 Incubation time	5	min	(1-15)	(default: 5)	
Bind 1 Settle time	10	min	(5-20)	(default: 10)	
Wash					
Wash volume	800	μL	(500-1000)	(default: 800)	
Wash Shake1 speed	1300	RPM	(800-1300)	(default: 1300)	
Wash Shake1 time	4	min	(1-10)	(default: 4)	
Wash Shake2 speed	1000	RPM	(800-1100)	(default: 1000)	
Wash Shake2 time	2	min	(1-10)	(default: 2)	
Wash Settle time	8	min	(5-15)	(default: 8)	
Ethanol_1					
•					•

Ethan	nol 2 cycles	2	time(s)	(1-3)	(default: 2)	*
Ethan	nol 2 volume	750	μL	(500-1000)	(default: 750)	
Ethan	nol 2 Settle time	4	min	(1-5)	(default: 4)	
Ethan	ol 2 Shake speed	800	RPM	(800-1200)	(default: 800)	
Ethan	nol 2 Dry time	5	min	(0-10)	(default: 5)	
Elution						
Elutio	n volume	40	μL	(20-100)	(default: 40)	
Elutio	n Shake speed	1000	RPM	(800-1200)	(default: 1000)	
Elutio	n Shake time	2	min	(1-15)	(default: 2)	
Elutio	on Incubation time	1	min	(0-5)	(default: 1)	
Elutio	on Settle time	2	min	(1-5)	(default: 2)	
Transfe	r 🖲 Yes 🗠 No					
Eluen	t Transfer volume	35	μL	(20-100)	(default: 35)	
Res	set to Default Values					
LIMS Pa	LIMS S	ettings				_
	,					
	Peltier	Offsets				
Lysis	Peltier temperature offset			40	°C	Ξ
DNas	e Peltier temperature offset			13	°C	
The <b>Reset</b> determined during met temperatu	to Default Values button does not reset d during installation and are meant to ensu hod execution. The actual Peltier temperal ure plus the Peltier temperature offset.	the Peltie re your sa ture will b	r temperat amples rea e equal to	ure offsets. Th ich the desired the sum of the	ese offsets are I temperature e <b>sample</b>	
•	m					F.

#### Figure 2.3 Default GUI Values, Bottom portion shown

- **5** Change or ensure that each variable is the correct default value. These values can be changed at run time. For details on this screen see Table 1.2.
- **IMPORTANT** Changing default protocol settings may cause invalid method results. Beckman Coulter's warranty applies only for unchanged Customer Default Protocol Settings.

To restore default settings, click the **Reset to Default Values** Button.

**IMPORTANT** The Reset to Default Values button does not reset the Peltier temperature offsets. These offsets are determined during installation and are meant to ensure your samples reach the desired temperature during method execution. The actual Peltier temperature is equal to the sum of the set temperature, plus the offset temperature. The formula would be as follows:

Sample Temp + Peltier Temp Offset = Actual Peltier Temp

For example, to determine the actual temperature of the Peltier for Lysis:

If the Lysis Temperature is set to 55°C, and the Lysis Peltier Temperature Offset is set to 40°C, the actual temperature of the Peltier would be:

```
55°C + 40°C = 95°C
```

**6** From the Biomek software, click **Execution > Run • Run**.

#### **Configuring the RNAdvance Blood Method**

The graphical user interface appears and allows you to make some selections. To achieve optimal performance for different sample types, you may need to adjust some selections.

The RNAdvance Blood Method contains a graphical user interface to ensure proper processing of samples. This interface contains four tabs, three of which step you through the setup process until the method is ready to run.

The four tabs include:

- **Quick Start** Use this Tab to configure the sample and finals plates, and to track reagent lot numbers. See the Quick Start Tab on page 2-6.
- **Columns** Use this Tab to define the number and location of active columns on the sample plates. See the Columns Tab on page 2-9.
- **Procedure** Use this Tab to configure the protocols. See the Procedure Tab on page 2-11.

**Recovery** — Use this Tab only if you need to restart the method and recover it at the point it was interrupted. See the Recovery Tab on page 2-18.

## **Quick Start Tab**

Figure 2.4 Quick Start Tab



- A. Quick Start Tab
- B. Input Values
- G. Dec
- C. Forward Button
- **D.** Labware Selector
- E. Output Values

- F. Tracking
- G. Deck Display
- H. Run Button
- I. Abort Button

Screen Element	Purpose	Do this	Notes
A Quick Start Tab	Allows you to define the sample inputs, outputs and whether to track the method.	Complete all fields.	N/A
B Input Values	Allows you to define the sample volume of the run.	Complete all fields.	N/A
C Forward Button	Allows you to advance to the next screen.	Click the button to advance.	Use this button or just click the next tab.
D Labware Selector	Allows you to determine the define the elution plate.	Select the desired elution plate type.	
E Output Values	Allows you to define the elution and transfer volumes.	Select whether to transfer to a final plate and then select the correct elution and (if transferring) transfer volumes.	Most laboratories choose to transfer the eluate to a new plate.
F Tracking	Allows you to track RNAdvance Blood reagents automatically by Lot numbers.	To track RNAdvance Blood reagents automatically, click Yes and record the User ID and the applicable lot numbers.	N/A
G Deck Display	Displays the correct deck setup based on your inputs.	Check the Deck Display against the Biomek 4000 Deck.	N/A
H Run Button	Allows you to start running the method.	Click the Run button.	This Button appears on all four tabs.
I Abort Button	Allows you to stop the method.	While the Application GUI is displayed, click this button to abort.	This Button appears on all four tabs.

Table 2.1	Describing the	<b>RNAdvance Blood</b>	Primary Screen

## To complete the Quick Start Tab Run Settings

- 1 On the Quick Start Tab select the sample volume in  $\mu L$ .
- $\label{eq:select_select} 2 \quad \text{Select whether to transfer the eluate to the final plate.}$

**NOTE** Most laboratories choose to transfer the eluate to a new plate.

**3** Click the Labware Selector. The Labware Selector screen displays. See Figure 2.5.

Figure 2.5 Labware Selector

Labware Type Selector
Change Labware
ElutionLabware Plate
ElutionLabware Plate Type
Type: ABgene_2800_FullSkirt_GSL
OK Cancel

- **4** Select the Elution Labware Plate Type used in your RNAdvance Method. Click **OK**.
- 5 If transferring, select the Elution and Transfer Volume in  $\mu$ L. The default volume that Beckman Coulter recommends is 40  $\mu$ L.
- **6** Select the Transfer Volume in  $\mu$ L. The default volume that Beckman Coulter recommends is 30  $\mu$ L.

**NOTE** Beckman Coulter recommends a transfer volume of at least 10µL less than the elution volume to avoid any bead carryover.

- 7 Select whether the application should write a log file. If **Yes**, follow the steps below. If **No**, skip to step 8.
  - a. Enter the User ID.
  - **b.** Enter the **RNAdvance Kit Lot** #.
  - **c.** Enter the **Elution Buffer Lot** #.
  - d. Enter the Ethanol Lot #
  - e. Enter the Isopropanol Lot #.
  - f. Enter the DNase Lot #.
  - **g.** Enter the **DNase Buffer Lot** #.
  - **h.** Enter **Notes** if needed.
  - **NOTE** If this feature is used, the RNAdvance Blood application will record a log file to the computer folder defined on the Configure Method screen. See Customer Default Settings on page 1-3 for information on how to access this screen.

8 Click the Columns Tab or the 찬 button. The Columns Tab Setup Screen is displayed.

#### **Columns Tab**

Figure 2.6 Columns Tab showing Sample Plate Selection





- C. 96-Well Plate Column
- B. Back Button

#### 🔨 WARNING

# Orbital shaker hazard. Distribute samples evenly across the plate to prevent the plate from becoming unbalanced. An unbalanced plate may propel hazardous substances from the orbital shaker. Refer to the *Biomek 4000 ALPs and Accessories Manual* for complete safety information.

Table 2.2 Describing the Columns Screen

Screen Element	Purpose	Do this	Notes
A Columns Tab	Allows you to define active columns.	Select columns in any order. Rows cannot be selected.	Any combination of individual columns can be made active, but cells cannot be made active as rows or as individual wells.
B Back Button	Allows you to return to the previous tab.	Select this button to return to the Quick Start Tab.	N/A
C 96-Well Plate	Allows you to visualize and select columns more easily.	Click on the heading buttons above the column to select and deselect columns to be run on the Biomek 4000.	Figure 2.6 shows all columns selected. Your application may be different.

#### To complete the Columns Tab

1 On the **Columns Tab**, select the active columns by clicking the appropriate 96-Well Plate Column Heading. To highlight all or none of the cells, click the top-left Well Plate Column Heading. This acts as a toggle.

**2** Click the **Procedure Tab** or the **D** button. The Procedure Tab Setup Screen displays.

#### **Procedure Tab**





- A. Procedure Tab
- B. Lock/Unlock Button
- C. Reset Default Values
- D. Procedure Values

 Table 2.3 Describing the Procedures Screen

Screen Element	Purpose	Do this	Notes
A Procedure Tab	Allows you to define and confirm all values in the RNAdvance Blood method.	Select all correct values. Changed values are highlighted in yellow.	The starting values are determined by the User Default and Settings step. See Customer Default Settings on page 1-3.
B Lock/Unlock Indicator	Indicates whether the method protocol inputs are locked with a password.	To lock the protocol values, click the lock tab and enter a password.	Only the values on the Procedures Tab are locked.
C Reset Default Values	Returns all values to the defaults established in the User Defaults and Settings step. See Customer Default Settings on page 1-3.	Click this button to return all values to the default values.	All manual entries will be lost without recourse.
D Procedure Values	Allows you to change values in the RNAdvance Blood method.	Select the correct procedure values. Changed values are highlighted in yellow.	For a list of all procedure value defaults, minimums and maximums, see Table 2.4.

The values for these steps originate from the Default Protocol Settings. You can access these settings from the User Defaults and Settings step. See Customer Default Settings on page 1-3.

Use this Tab to customize the automated protocol at runtime. Most RNAdvance Blood variables can be adjusted. All protocol settings are written to memory when the run starts and recalled for subsequent runs.

Table 2.4 lists all automated method steps and the default, minimum and maximum values.

Step	Step Description	Units	Default	Minimum	Maximum
Lysis					
1	Lysis Volume	μL	300	100	500
2	Proteinase K Solution Volume	μL	40	20	100
3	Lysis Shake speed on the Orbital Shaker	RPM	1200	1000	1200
	Lysis Shake time	minutes	3	1	60
4	Lysis Sample temperature <sup>a</sup>	°C	55	20	100
	Lysis Incubation time	minutes	25	1	60
Bind 1	I				
5	Bind 1 Volume	μL	410	200	500
6	Bind 1 Tipmixing	minutes	15	1	20
7	Bind 1 Incubation time	minutes	5	1	15
8	Bind 1 Settle time	minutes	10	5	20
Wash					

 Table 2.4
 Procedure Default, Minimum and Maximum Values

Step	Step Description	Units	Default	Minimum	Maximum
9	Wash Volume	μL	800	500	1000
10	Wash Shake 1 speed on the Orbital Shaker	RPM	1300	800	1300
11	Wash Shake 1 time	minutes	4	1	10
12	Wash Shake 2 speed on the Orbital Shaker	RPM	1000	800	1100
13	Wash Shake 2 time	minutes	2	1	10
14	Wash Settle time	minutes	8	5	15
Ethan	ol 1				
15	Ethanol 1 Volume	μL	750	500	1000
16	Ethanol 1 Shake speed	RPM	1100	800	1200
17	Ethanol 1 Shake time	minutes	2	1	5
18	Ethanol 1 Settle time	minutes	3	1	10
19	Ethanol 1 Dry time	minutes	5	1	15
DNase	2				
17	DNase Solution Volume	μL	100	20	150
19	DNase Shake speed on the Orbital Shaker	RPM	1000	800	1200
	DNase Shake time	minutes	2	1	5
20	DNase Sample Temperature <sup>b</sup>	°C	37	20	100
	DNase Incubation time	minutes	15	1	20
Rebin	d with Bind 2				<b>.</b>
21	Bind 2 Volume	μL	200	100	500
22	Bind 2 Shake Speed on the Orbital Shaker	RPM	1000	800	1200
	Bind 2 Shake time	minutes	8	1	10
23	Bind 2 Incubate time	minutes	5	1	10
24	Bind 2 Settle time	minutes	5	1	10
Ethan	Ethanol 2				
25	Ethanol 2 Cycles	time(s)	2	1	3
26	Ethanol 2 Volume	μL	750	500	1000
27	Ethanol 2 Settle time	minutes	4	1	5
28	Ethanol 2 Shake Speed on the Orbital Shaker	RPM	800	800	1200
29	Ethanol 2 Dry time	minutes	5	0	10
Elution					
30	Elution volume	μL	40	20	100

Table 2.4 Procedure Default, Minimum and Maximum Values (Continued)

Step	Step Description	Units	Default	Minimum	Maximum
31	Elution Shake speed on the Orbital Shaker	RPM	1000	800	1200
32	Elution Shake time	minutes	2	1	15
33	Elution Incubation time	minutes	1	0	5
34	Elution Settle time	minutes	2	1	5
Transfer					
35	Eluent Transfer volume	μL	35	20	100

 Table 2.4 Procedure Default, Minimum and Maximum Values (Continued)

a.Set Lysis Sample Temperature to the desired Temperature in the well. See D Peltier Offsets in RNAdvance Blood Customer Default Values.

b.Set DNase Sample Temperature to the desired Sample Temperature in the well. See D Peltier Offsets in RNAdvance Blood Customer Default Values.

**IMPORTANT** Changing default protocol settings may cause undesirable results. Beckman Coulter's warranty applies only for unchanged Default Protocol Settings.

#### To complete the Procedures Tab

1 On the **Procedures Tab** change or ensure that each variable is correct. Use the up and down arrow keys to select values. A value with a yellow background indicates that the value has changed from the default values.

**2 Optional:** To lock the values of the procedure, which prevents inadvertent changes to variables,

click the Lock/Unlock Button <a>[</a>. The Lock/Unlock screen displays.

Figure 2.8 Lock/Unlock Password

New Password			
Enter Current Password:			
Enter New Password:			
Re-Enter New Password:			
OK Cancel			

**NOTE** For first-time use, leave the first field, **Enter Current Password**, blank.

- a. Enter a new password.
- **b.** Re-enter the password.

**NOTE** To change the default values so that the variables here populate according to custom values, see Customer Default Settings on page 1-3.

**c.** Click **OK**. All variables become non-editable. To change locked values, click the Lock/Unlock Button and enter the password.

**NOTE** If a password is forgotten, contact Reagent Support at *reagentsupport@beckman.com*.

**3 Optional:** To reset all variables to the default values, click the

Reset Default Values Button 🗟 . All changed variables revert to default values.

Once the method begins running the software prompts you to set up reagents, tools and labware. See Table 2.5 for the sequence of this setup. Follow the prompts on the screen for actual values.





#### **Running the RNAdvance Blood Method**

**IMPORTANT** To ensure best possible outcomes, the RNAdvance Blood bottle containing Bind 1 must be shaken and mixed so that the beads are homogenous in solution. Run the procedure immediately following the placement and filling of RNAdvance Blood reagents. Failure to run the method immediately following the filling of reagents may cause the method to produce poor results.

After entering all protocol variables, positioning labware, and filling all reagent reservoirs, the RNAdvance Blood Method is ready to run.

#### To run the RNAdvance Blood Method

- **1** Confirm that all values in the user interface are correct for all RNAdvance Blood application variables. See Configuring the RNAdvance Blood Method.
- **2** From any of the user interface tabs, click the green run arrow **P**.

Step	Reagent/Tool/ Labware type	Labware Name	Notes
Mix	DNase	N/A	Nuclease Free Water
Reagents			DNase I buffer
			DNase I
			Use freshly prepared DNase I solution
	Ethanol	N/A	Use freshly prepared 70% ethanol
Put Tools	Gripper	N/A	N/A
on the Deck	MP1000	N/A	N/A
Put	p1000 Barrier Tips	ReagentTips	N/A
Labware on the Deck		SampleTips_1	
		BloodTips	
	p200 Barrier Tips	ElutionTips_1	N/A
	96R Super Magnet Plate	Mag_1	N/A
	p1000 Barrier Tips	SampleTips_1	N/A
	96-well plate	ElutionPlate_1	N/A
	Half Modular Res-Bind- Reservoir WashBuffer	Res-Bind-	Bind 1 in Section 1
		WashBuffer	Washbuffer in Section 2
	AGCT_96RitterDeep Square_GSL	PrepPlate1_1	Sample in selected columns 1-8
	RNABlood_Mod_Res Re RE	Res-LY-PK-DN-	Lysis in section 1
		RB-EL	PK in section 2
			DNase in section 3
			Bind2 in section 4
			Elute in section 5
			Sections 6 and 7 are empty
	IMReservoir96	Res-Ethanol	Ethanol in section 1

Table 2.5 Reagent and Deck Setup Guide

The Biomek instrument may appear to be idle at times, during which a timer appears. When this occurs an incubation time is being observed before the next step can be performed. See Figure 2.10.

#### Options for the timer include

- Editing the timer duration to the precise number of hours, minutes and seconds.
- Adding one minute to the elapsed time.
- Expiring the timer immediately.

#### Figure 2.10 Editing or Expiring the Timer

Incubation Timer Bind1AddInc PrepPlate_1				
Current Time:	11:21:47 PM			
Timer Duration:	00:05:00			
Time Left:	00:03:06			
	Begin End			
	Edit Timer Add 1 Minute Expire Now			

When the method ends, the tips are unloaded and the Biomek Start arrow returns to green.

## **Recovering from an Interrupted Method**

There are several reasons a method may be interrupted:

- Power failure
- Tips did not load properly
- Insufficient reagent
- Instrument crash

The RNAdvance Blood Method provides ways to recover or restart the method efficiently:

- Restart from the point where the method stopped.
- Reset all steps for the plate.
- Reset the deck: return all moveable labware to their starting positions.

#### **Recovery Tab**

Figure 2.11 Recovery Tab



- A. Recovery Tab
- B. Plate Column
- **C.** Reset Current Selection
- D. Reset Current Plate
- E. Set All Sections of Current Plate to Match Current Selection
- F. Finished Tasks
- G. Unfinished Tasks
- H. Reset the Deck

Screen Element	Purpose	Do this
A Recovery Tab	Allows you to see where the RNAdvance Blood Method was interrupted to more efficiently recover it.	Use this tab to reset the specific columns to be run again.
B Plate Column	Allows you to visualize the individual columns of the Plate.	Click the column tab to check the status of each column.
C Reset Current Selection	Allows you to restart the current column to the beginning of the method.	Click this button to restart the selected column to the beginning of the method.
D Reset Current Plate	.Allows you to restart the method from the beginning.	Click this button to restart the RNAdvance Blood method from the beginning.
E Set All Sections of Current Plate to Match Current Selection	Allows you to set all active columns to the state of the currently selected column.	Click this button to set all active columns to start from the state of the currently selected column.
F Finished Tasks	Cleared checkboxes show completed tasks by column.	To rerun a step for a column, check the appropriate checkbox.
G Unfinished Tasks	Checked checkboxes show unfinished tasks. The green bar indicates the task next in line.	To skip a step for a column, clear (uncheck) the appropriate checkbox.
H Reset the Deck	Moves all labware to the starting position.	Click this button to show how the deck should be reset.

#### Table 2.6 Describing Recovery Tab

#### To Recover the RNAdvance Blood Method

- **1** Address the source of the interruption.
- **2** Restart the RNAdvance Blood Method. The **Recovery** tab will be displayed.
- **3** Confirm that the state of the deck on the Biomek 4000 matches the Deck Display. If the deck on the Biomek 4000 does NOT match the Deck Display, correct the deck on the Biomek 4000 to match it by dragging labware in the Deck Display.
- **4** Click any Tasks that show finished, but need to be recovered.

For example, if you can see that column eight does not have Bind 1 reagent, but the Recovery Tab shows that it was added, click that task in the Column 8 tab to make it an unfinished task.

**5** Click the **Run** button

#### Method Operation

Operating the RNAdvance Blood Method

# Abbreviations

- $\mu$ L microliter
- ALP Automated Labware Positioner
- DNA Deoxyribonucleic Acid
- GUI Graphical User Interface
- mL milliliter
- LIMS Laboratory Information Management System
- **m** meter
- RNA Ribonucleic Acid
- SPRI Solid Phase Reverse Immobilization
- Vol Volume

Abbreviations

# Glossary

assay - procedure of repeat testing to determine the assigned value for a given lot and level of control.

- beads in SPRI technology, magnetic, uniform microparticles.
- **dead volume** in an automated system, the amount or volume of a sample or reagent that cannot be picked up by the pipette tip.
- elution buffer buffer which elutes DNA from magnetic particles.
- ethanol wash washes the magnetic beads with 85% ethanol to remove contaminants.
- **gripper** a tool that includes mechanical fingers to grip labware. Gripper fingers grasp labware along the long side and move the labware from one location on the Biomek deck to another. The gripper contains two fingers: a double gripper located to the front and a single gripper located to the back of the tool.
- PAXgene Preservative and stabilizer for nucleic acids in whole blood.
- reservoir one-well labware receptacle holding liquid to be used in a method.
- samples PAXgene preserved human blood.
- **supernatant** a liquid lying above a solid residue after crystallization, precipitation, centrifugation, or other process.

Glossary

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