Instructions For Use

Biomek 4000

RNAClean XP Application

BECKMAN COULTER

Biomek 4000 RNAClean XP Application
Instructions for Use
PN B45820AA (March 2014)

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, March 2014
Biomek Software version 4.1 or higher

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**

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

⚠ **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.

B45820AA

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Introduction

Intended Use

This 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 RNAClean XP method. It contains information on:

- Instrument Requirements
- Method Operation

About This Manual

NOTE Screens in this manual may differ slightly from the screens in your RNAClean XP 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 RNAClean XP method.

Chapter 2, Method Operation
Contains the settings and procedures for operating the RNAClean XP 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

Instrument Requirements

The RNAClean XP application requires a Biomek 4000 with software version 4.1 or higher. Contact your Beckman Coulter Representative for the method installation on 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 and Figure 1.2 for the recommended deck layout for an 8-position deck and a 12-position deck.

NOTE A one-plate method can be run on either an 8-position or 12-position deck, but two-plate methods can only be run on a 12-position deck.

Figure 1.1 Recommended deck layout for an 8-position deck
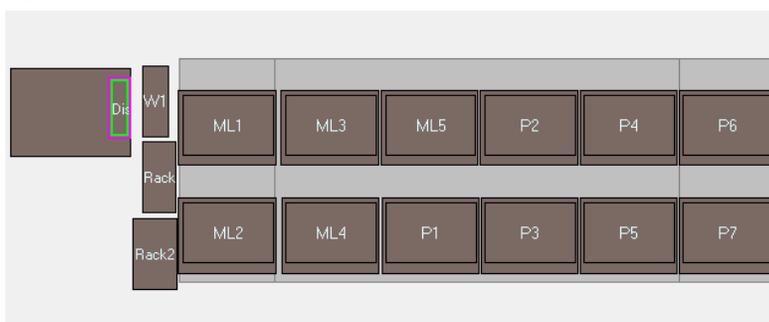

Figure 1.2 Recommended deck layout for a 12-position deck

Table 1.1 Requirements for the RNAClean XP Method

Type	Qty for 1-plate run	Qty for 2-plate run	Description	Part number	Manufacturer
Tools	1	1	Gripper Tool	987371	Beckman Coulter
	1	1	MP200 Eight-Tip Pipette Tool	986146	
	0	1	Left Side Module	987264	
	0	1	Right Side Module	987263	
ALPs	1	1	Liquid Waste ALP	B21398	Beckman Coulter
	1	1	Disposal ALP	609751	
	4	7	Labware Holder, Gray	609120	
	3	5	Tip Rack Holder	391910	
	0	1	Off Deck Gripper Rack	B21400	
	1	1	Liquid Waste Disposal Kit	B21398	
	0	1	Off Deck Tool Rack Kit (left)	B21395	
	1	0	Gripper Tool Rack	609641	
Magnet Plate	1	2	SPRIPlate 96R Super Magnet Plate	A32782	Beckman Coulter
Reservoirs	1	1	Reservoir Frame	372795	Beckman Coulter
	1	1	Half Reservoir	372786	
	2	2	Quarter Reservoir	372790	
Consumables	3	5	Biomek AP96 P250 Tips, Pre-Sterile with Barrier	717253	Beckman Coulter
	2	4	96 Well PCR Plate (semi-skirted)	AB-1400[a]	Thermo Scientific
	2	4	96 Well Costar 3797 (used as a support plate for semi-skirted plates)	3797	Costar
User-Supplied Reagents	NA	NA	RNAClean XP Kit	A63987, A66514	Beckman Coulter
	NA	NA	70% Ethanol, undenatured	Ethanol supplier	Ethanol supplier
	NA	NA	Nuclease-free water	AM9932	Life Technologies

a. ABGene 1400 or equivalent.

Installation

Your Beckman Coulter Field Applications Specialist performs all Biomek and
RNAClean XP Application installation and setup services. For service, see your Beckman Coulter
Representative.

Method Operation

Configuring the RNAClean XP Method

Establishing Configuration Settings

The RNAClean XP 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 RNAClean XP method application.

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

Application 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 RNAClean XP method by clicking **File > Open.** The method opens and the method outline displays. See Figure 2.1.

> **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 Configure method outline
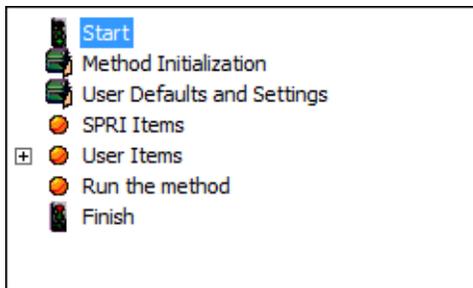
3 From the method outline click **User Defaults and Settings.** See Figure 2.2. The screen in Figure 2.3 displays.

Figure 2.2 Method outline, User Defaults and Settings highlighted
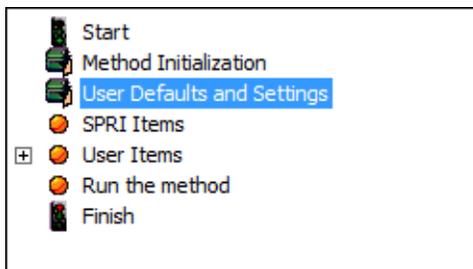
4 Recommended default values are displayed, which can be modified as needed.

> **NOTE** The **Supernatant and Wash Removal Height** should only be adjusted by your field application specialist. Changing this value may result in inadequate supernatant removal and poor results.

Figure 2.3 Application default values

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 Default settings, click **Reset to Default Values**.

5 Enter the **LIMS path** as needed.

6 Change or ensure that each variable is the correct default value. These values can be changed in the Graphical User Interface (GUI) at run time. For details on this screen see Table 2.1.

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

Table 2.1 RNAClean XP Customer Default Value descriptions

Screen Element	Purpose	Action
A Default Protocol Settings	These values can be adjusted for customer specified processes. These values will feed into the application GUI as the default values.	Change values as needed based on your specific protocol.
B Reset to Default Values	Replaces all values, back to protocol default values.	Click the button to revert all values to the default values.
C Supernatant and Wash Removal Height	Sets the precise pipetting height within the well for optimal supernatant and waste removal steps.	The **Supernatant and Wash Removal Height** should only be adjusted by your field applications specialist. Changing this value may result in inadequate supernatant removal and poor results.
D 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: `c:\users\public\documents\SPRI\RNACleanXPB4K\RNACleanLogs`

Operating the RNAClean XP Method

The RNAClean XP method contains a Graphical User Interface (GUI) to ensure proper processing of samples. After starting the method, the GUI appears and allows you to make specific selections for the current run. To achieve optimal performance for different sample types, you may need to adjust some default selections.

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 final 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 2-10.

Procedure — Use this Tab to modify the current run parameters from the defaults. See the Procedure Tab on page 2-12.

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	**F.**	Tracking
B.	Input Values	**G.**	Deck Display
C.	Forward Button	**H.**	Run Button
D.	Labware Selector	**I.**	Abort Button
E.	Output Values		

Table 2.2 Quick Start screen information

Screen Element	Purpose	Action	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 number of plates and sample volume of the run.	Complete all fields.	N/A
C Forward Button	Allows you to advance to the next tab.	Click the button to advance.	NA
D Labware Selector	Allows you to define the sample plate and final elution plate.	Select the desired labware to be used for the sample plate and final plate.	When selecting a semiskirted plate, a support base plate will be required to support the plate on the Biomek Deck.
E Output Values	Allows you to define the elution and transfer volumes.	Select whether to transfer to a final plate and then select the desired elution and transfer volumes (if applicable).	Final eluate can remain in the sample processing plate (with the beads) to reduce consumable use.
F Tracking	Allows you to track the RNAClean XP reagents by lot number.	To track RNAClean XP reagents, click **Yes** and record the User ID and the applicable lot numbers.	The RNAClean XP application will record a LIMS log file to the computer folder defined in the LIMS path. See Figure 2.3.
G Deck Display	Displays the correct deck setup based on the 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.

To complete the Quick Start Tab

1 On the **Quick Start Tab** select the number of plates to run and the sample volume in μL.

2 Select whether to transfer the eluate to the final plate.

3 Select the Elution Volume in μL. The default volume that Beckman Coulter recommends is 40 μL.

4 If transferring, select the Transfer Volume in μL. The default volume that Beckman Coulter recommends is 35 μL.

NOTE Beckman Coulter requires a transfer volume of at least 5 μL less than the elution volume to avoid any bead carryover.

5 Click **Labware Selector**. The Labware Selector screen displays. See Figure 2.5.

IMPORTANT When selecting a semiskirted plate, a support base plate will be required to support the plate on the Biomek Deck.

Figure 2.5 Labware Selector
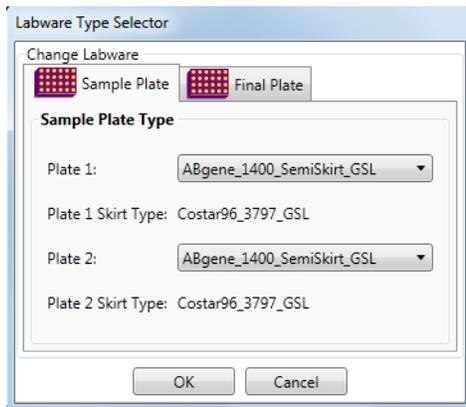
6 Select the desired **Sample Plates** and **Final Plates** to use in the RNAClean XP method. Click **OK**.

NOTE Additional labware may be supported with help from your local Beckman Coulter Field Applications Scientist.

The following is a list of default sample and final plate types:

- AB_1127
- ABgene _1100_SemiSkirt_GSL
- **ABgene _1400_SemiSkirt_GSL** (default)
- ABgene _1900_SemiSkirt_GSL
- ABgene _2800_SemiSkirt_GSL
- ABI_SemiSkirt_Microamp_Optical96_GSL
- BCI_thermo96_SemiSkirt
- BioRad_HardShell_FullSkirt_GSL
- BioRad_SemiSkirt96_HSS_GSL

7 Select whether the application should write a LIMS log file. If **Yes**, follow the steps below. If **No**, skip to step 8.

    a. Enter the **User ID**

    b. Enter the **RNAClean XP** Kit Lot #

    c. Enter the **70% Ethanol** Lot #

    d. Enter the **Elution Buffer** Lot #

    e. Enter **Notes** if needed

    NOTE If this feature is used, the RNAClean XP application will record a log file to the computer folder defined on the User Defaults and Settings screen. See Application Default Settings for information on how to access this screen.

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

Columns Tab

Figure 2.6 Columns Tab showing sample column and plate selection
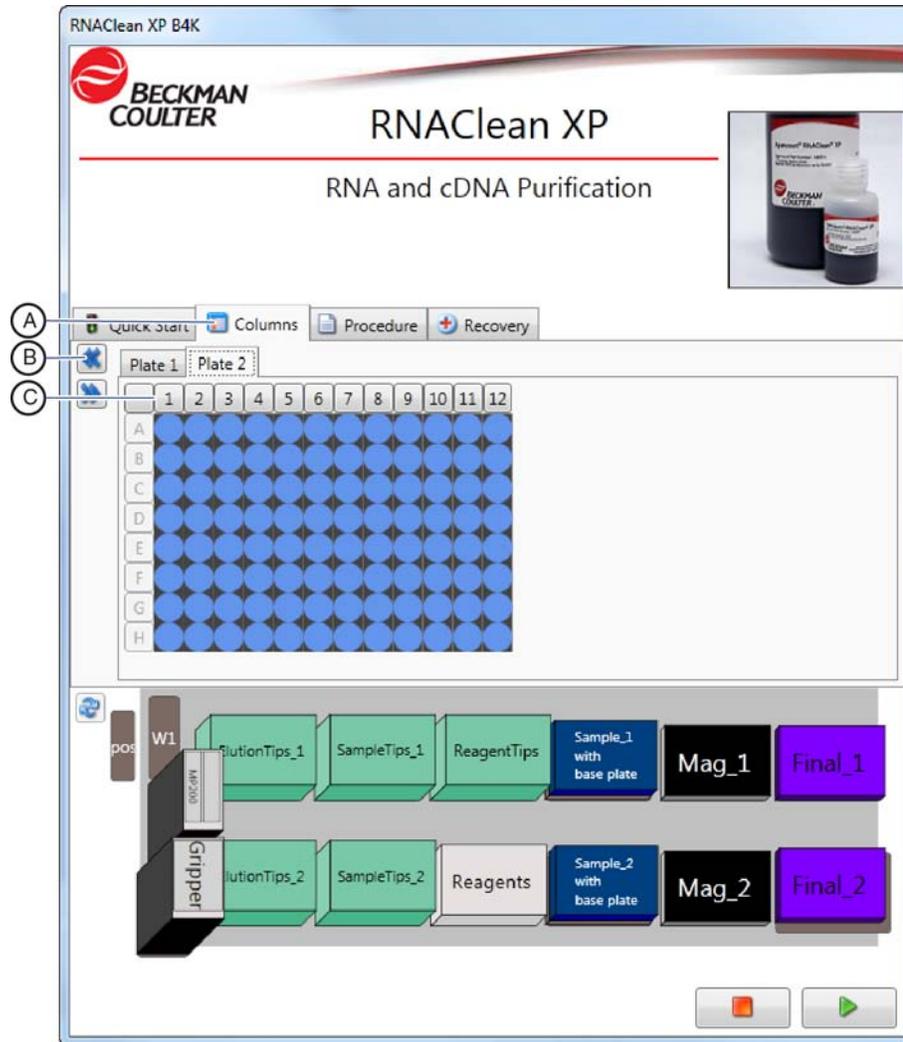
A. Columns Tab   **C.** 96-Well Plate Column Selector

B. Back Button

Table 2.3 Columns screen information

Screen Element	Purpose	Action	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.

Table 2.3 Columns screen information *(Continued)*

Screen Element	Purpose	Action	Notes
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 Column Selector	Allows you to visualize and select columns more easily.	Click the heading buttons above the column to select and deselect columns to be run.	Figure 2.6 shows all columns selected. Your specific run 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 corner of the column heading. This also acts as a toggle.

2 If the run includes two plates, select the active columns in the second plate.

3 Click the **Procedure Tab** or the ⏩ button. The Procedure Tab Setup Screen displays.

Procedure Tab

Figure 2.7 Procedure Tab
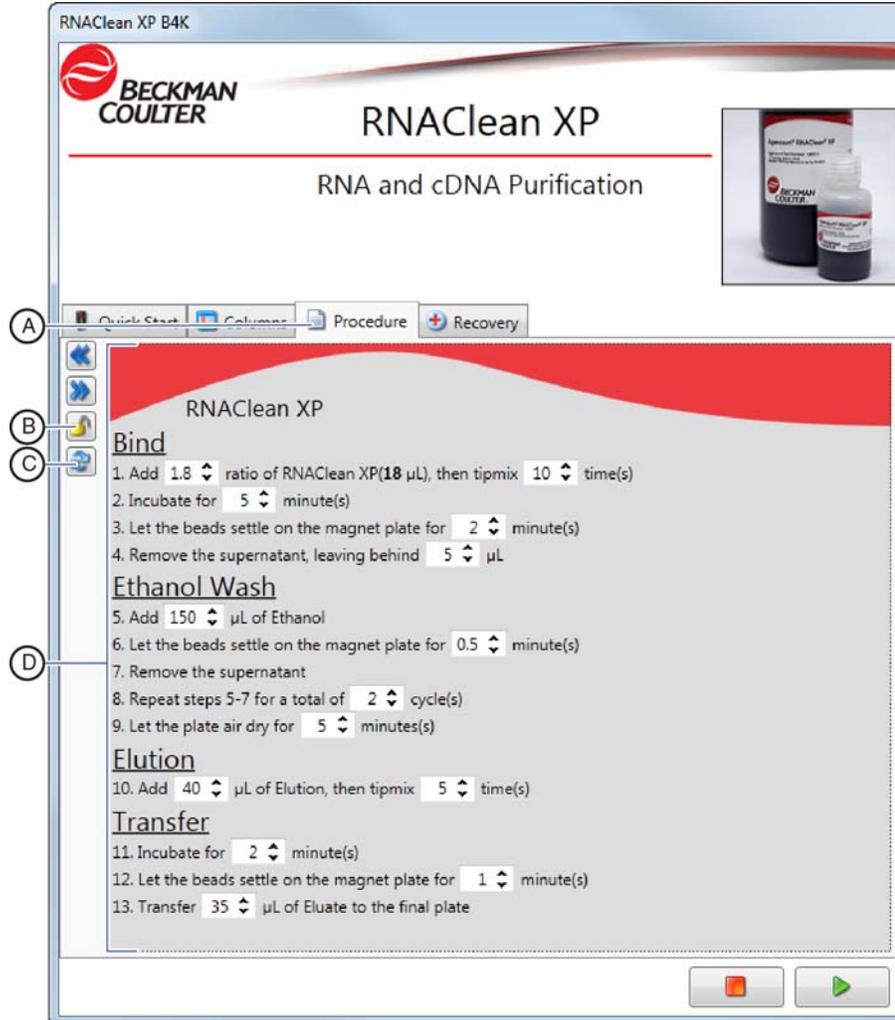
A. Procedure Tab
B. Lock/Unlock Button
C. Reset Default Values
D. Procedure Values

Table 2.4 Procedure screen information

Screen Element	Purpose	Action	Notes
A Procedure Tab	Allows you to define and confirm all values used in the RNAClean XP method.	Select all correct values. Values that differ from the defaults are highlighted in yellow.	The starting values are determined by the User Defaults and Settings step. See Application Default Settings.
B Lock/Unlock Indicator	Indicates whether the method protocol inputs are locked with a password.	To lock the protocol values, click the lock button and enter a password. See Figure 2.8.	Only the values on the Procedure Tab are locked.

Table 2.4 Procedure screen information *(Continued)*

Screen Element	Purpose	Action	Notes
C Reset Default Values	Returns all values to the defaults established in the User Defaults and Settings step. See Application Default Settings.	Click this button to return all values to default values.	All manual entries will be lost.
D Procedure Values	Allows you to change procedure settings in the RNAClean XP method.	Select the correct procedure values. Values changed from the defaults are highlighted in yellow.	All protocol settings are written to memory when the run starts and then recalled for subsequent runs. For a list of all procedure value defaults, minimums and maximums, see Table 2.5.

Table 2.5 lists all automated method steps and the default, minimum and maximum values.

Table 2.5 Procedure default, minimum and maximum values

Step	Step Description	Units	Default	Minimum	Maximum
Bind					
1	RNAClean XP/Sample Ratio	N/A	1.8	0.1	3
2	Bind Tipmixing	times	10	1	100
3	Bind Incubation time	minutes	5	0	30
4	Bind Settle time	minutes	2	0	15
5	Leave Residual volume	µL	5	0	200
Ethanol Wash					
6	70% Ethanol Volume	µL	150	1	200
7	Wash Counts	cycles	2	0	5
8	Wash Incubation time	minutes	0.5	0	5
9	Dry time	minutes	5	0	30
Elution					
10	Elution Buffer Volume	µL	40	40	200
11	Elution Tipmixing	times	5	1	25
Transfer					
12	Elution Incubation time	minutes	2	0	15
13	Elution Settle time	minutes	1	0	15
14	Elute Transfer Volume	µL	35	15	200

To complete the Procedure Tab

1 On the **Procedure 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.

> **NOTE** To change the default values so that the variables here populate according to custom values, see Application Default Settings.

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

2 **Optional:** To lock the values of the procedure, which prevents inadvertent changes to variables, click the **Lock/Unlock Button** . The Lock/Unlock screen displays.

Figure 2.8 Lock/Unlock password

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

  a. Enter a new password.

  b. Re-enter the password.

  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* for help with resetting it.

3 **Optional:** To reset all variables to the default values, click the Reset Default Values Button . All changed variables revert to default values.

Running the RNAClean XP Method

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

To run the RNAClean XP Method

1 Confirm that all values in the user interface are correct for all RNAClean XP application variables. See Operating the RNAClean XP Method.

2 From any of the user interface tabs, click the green run arrow .

**3** Once the method begins running, the software prompts you to set up reagents, tools and labware. See Table 2.6 for the sequence of this setup. Follow the prompts on the screen for automatically calculated volumes and the reservoir location for the reagent.

Table 2.6 Reagent and deck setup guide, example of a 1-plate run

Step	Reagent/Tool/ Labware type	Labware Name	Notes
Mix Reagents	70% Ethanol	N/A	Use freshly prepared 70% ethanol
Put Tools on the Deck	Gripper	N/A	N/A
	MP200	N/A	N/A
Put Labware and Reagents on the Deck	AP96_200μL_Barrier	ReagentTips SampleTips_1 Elute_Tips_1	N/A
	SuperMag	Mag_1	N/A
	RNAClean XP	Reagents	Ensure that the RNAClean XP reagent is mixed thoroughly and place it on the deck immediately prior to executing the method.
	Nuclease-Free Water		N/A
	70% ethanol		N/A
	ABgene_1400_Semi Skirt_GSL	Sample_1	Stack on top of a base plate of type Costar96_3797_GSL
	ABgene_1400_Semi Skirt_GSL	Final_1	Stack on top of a base plate of type Costar96_3797_GSL

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 2.9.

Figure 2.9 Editing or expiring the timer
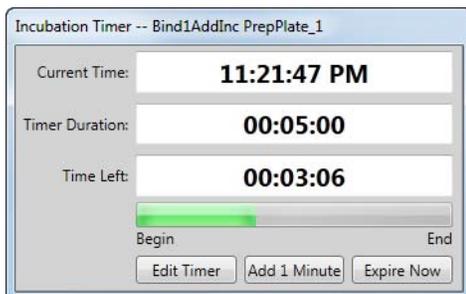
Options for the timer include

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

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 RNAClean XP 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.

At the start of a new run, all the tasks are highlighted in green and checked with green check marks. This indicates that all tasks are set to run. If the method was started but interrupted before its completion, the completed tasks become red completion symbols, while incomplete tasks still have green check marks. The darker green horizontal bar indicates the task next to be completed when the method resumes.

The process of recovering an interrupted method can be divided into four steps:

1. Determine the cause of the error and correct it.
2. Visually inspect the deck and labware to see if the status is as expected. Then manually adjust the deck as needed.
3. Determine where to restart the method on the Recovery Tab screen.
4. Adjust the deck display to match the physical deck.

   NOTE You may want to repeat a task or a series of tasks even though a task was completed (this depends on how and why the method was interrupted). If this is the case, make sure that the tasks in each column to be repeated are green, with the next task to be run, highlighted dark green.

Recovery Tab

Figure 2.10 Recovery Tab
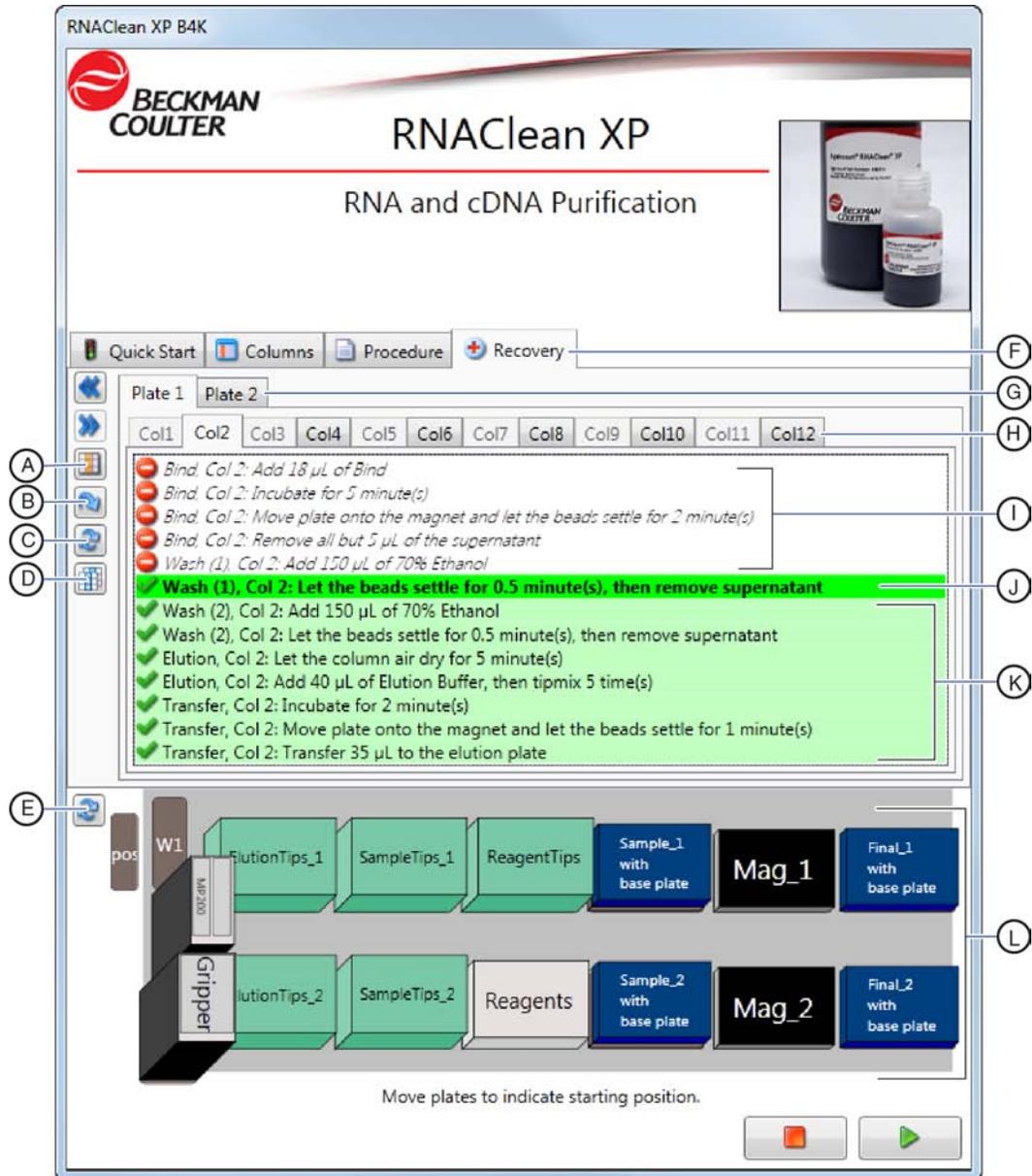
A. Reset Current Selection	**F.** Recovery Tab
B. Reset Current Plate	**G.** Plate Tab
C. Reset All Plates	**H.** Plate Column
D. Set All Sections of Current Plate to Match Current Selection	**I.** Finished Tasks
	J. Next Task to be completed
	K. Unfinished Tasks
E. Reset the Deck	**L.** Deck Display

Table 2.7 Recovery screen information

Screen Element	Purpose	Action
A 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.
B Reset Current Plate	Allows you to restart the method from the beginning for the plate selected.	Click this button to restart the current plate to the beginning.
C Reset All Plates	Allows you to restart the method from the beginning for both plates.	Click this button to restart the RNAClean XP method from the beginning.
D Set All Sections of Current Plate to Match Current Selection	Allows you to set all active columns at once 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.
E Reset the Deck	Moves all labware to the starting position.	Click this button to show how the deck should be reset.
F Recovery Tab	Allows you to see where the RNAClean XP method was interrupted to more efficiently recover it.	Use this tab to reset the specific columns to be run again.
G Plate Tab	Allows you to see plate 1 or plate 2 status.	Click the plate tab.
H Plate Column	Allows you to visualize the individual columns of the Plate.	Click the column tab to check the status of each column.
I Finished Tasks	Completion symbols show completed tasks by column.	To rerun a step for a column, check the appropriate checkbox. It turns from red to green.
J Next Task to be completed	Allows you to see the task next to be completed when the method resumes.	N/A
K Unfinished Tasks	Green checkboxes show unfinished tasks.	To skip a step for a column, clear (uncheck) the appropriate checkbox.
L Deck Display	Allows you to see how the RNAClean XP method tracks the deck. Also allows you to move labware as needed.	If the labware does not match the physical Biomek 4000 deck, click and drag the labware appropriately.

To Recover the RNAClean XP Method

1 Address the source of the interruption.

2 Restart the RNAClean XP Method. The **Recovery** tab will be displayed. The screen shows the place that the method stopped. Red tasks show what has been completed; green tasks show what is incomplete. The deck display shows the current placement of labware.

3 Visually inspect the physical deck and labware to see if the status is as expected. Then manually adjust the deck as needed.

4 Determine where to restart the method on the Recovery Tab.

For example, if you can visually see that column 8 of the plate does not have RNAClean XP reagent, but the Recovery Tab shows that it was added, click that task in the Column 8 tab to make it an unfinished (green) task.

NOTE If tasks are to be repeated, ensure that enough reagent volume is in the reservoirs to complete the run.

5 Click the **Run** button .

Abbreviations

µL — microliter

ALP — Automated Labware Positioner

cDNA — complementary DNA

cm — centimeter

DNA — deoxyribonucleic acid

GUI — Graphical User Interface

mm — millimeter

LIMS — Laboratory Information Management System

RNA — ribonucleic acid

SPRI — Solid Phase Reversible Immobilization

beads — in SPRI technology, paramagnetic, uniform microparticles.

eluate — The final purified solution of solvent and nucleic acids resulting from elution.

elution — The process of extracting a substance that is adsorbed to another by exposing it to a solvent — in SPRI technology, separating the nucleic acids from the SPRI beads, usually with nuclease-free water or Tris solution.

elution buffer — buffer which elutes DNA from SPRI beads, such as nuclease-free water or other elution buffers.

ethanol wash — washes the SPRI beads with 70% 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.

reservoir — one-well labware receptacle holding liquid to be used in a method.

SPRI technology — Solid Phase Reversible Immobilization; bead technology used to purify nucleic acids.

supernatant — a liquid lying above a solid residue after crystallization, precipitation, centrifugation, or other process.

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