

QuantiFluor® Dye Quantitation Methods for the Maxprep® Liquid Handler

Instructions for Use of Products AS9100, AS9101, AS9105, AS9200, AS9201 and AS9205



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All technical literature is available at: www.promega.com/protocols/

Visit the website to verify that you are using the most current version of this Technical Manual.

Email Promega Technical Services if you have questions on use of this system: techserv@promega.com

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Description

The Maxprep® Liquid Handler automates the post-processing setup of QuantiFluor® Dye Quantitation reactions including serial dilution of standard curves, dilution of samples and placement of controls. The QuantiFluor® Dye Quantitation protocol allows the flexibility for administrators to create variant methods in the Maxprep® software that specify quantitation reaction setup options to meet the needs of the laboratory. All of the QuantiFluor® systems (QuantiFluor® dsDNA System, QuantiFluor® ONE dsDNA System, QuantiFluor® RNA System and QuantiFluor® ssDNA System) can be used with the QuantiFluor® Dye Quantitation method. When starting a method, you can indicate the input and output labware for the reaction and whether the samples should be diluted 100-fold prior to quantitation setup.

The Maxprep® Liquid Handler accepts deck trays from the following Maxwell® instruments as input labware.

Instrument	Deck Tray Type	Catalog Number
Maxwell® RSC	16-position	AS4500
Maxwell® FSC	16-position	AS4600
Maxwell® CSC ¹	16-position	AS6000
Maxwell® RSC 48	24-position	AS8500
Maxwell® CSC 48 ¹	24-position	AS8000

¹The Maxprep® Liquid Handler is compatible with the Maxwell® CSC Instruments when operated in the research use only (RUO) mode.

2 Before You Begin

2.1 Maxprep® Liquid Handler QuantiFluor® Dye Quantitation Method

As new Maxprep® Liquid Handler methods are released, they will be listed on the Promega web site and available for download at: www.promega.com/resources/software-firmware/maxprep-liquid-handler-methods/

This document presents guidelines for interaction with the post-processing QuantiFluor® Dye Quantitation method on the Maxprep® Liquid Handler. Reaction setup, placement of controls and standard curve settings for quantitation setup are specified in variant methods and cannot be altered at run time. After selecting a variant method to run, you will enter processing parameters (e.g., Input Labware, Output Labware, Sample Dilution) and be led through a series of user interface screens that detail placement of labware and reagents on the Maxprep® Liquid Handler. The Maxprep® Liquid Handler is provided with a PC and touch-screen monitor. Interactions with the user interface screen can occur using the mouse and keyboard, touch gestures on the monitor or a combination of both approaches.

2.2 Materials to be Supplied by the User

- black 96-well flat bottom assay plate (e.g., Corning, Cat.# 3915)
- strip tubes (user-specified , e.g., Thermo Fisher MicroAmp™ 8-Tube Strip, 0.2 ml; for standard curve preparation)
- amplification plate base (e.g., Thermo Fisher MicroAmp® 96-well base, Cat.# N8010531; for strip tubes)
- Maxprep® Reagent Reservoir, 60ml (Cat.# AS9304)
- Maxprep® 50µl Conductive Disposable Tips, Filtered (Cat.# AS9301)
- Maxprep® 300µl Conductive Disposable Tips, Filtered (Cat.# AS9302)
- **optional:** 1.1ml, Square-Well V-Bottom Deep Well Plate (Cat.# V6821; for dilution prior to quantitation setup)

2.3 Selecting a Maxprep® Liquid Handler QuantiFluor® Dye Quantitation Method

To start a method on the Maxprep® Liquid Handler, perform the following steps:

1. Start the Maxprep® software by double-clicking the Maxprep® software icon on the Windows® desktop. Alternatively, select the **Windows Start** button in the lower left corner of the screen, choose **All Programs**, open the Promega folder and select the **Maxprep** software icon.
2. From the Maxprep® software 'Home' screen (Figure 1), press the **Start** button.



Figure 1. Maxprep® software 'Home' screen. From the Maxprep® software 'Home' screen, select the **Start** button to begin the process of running a method.

3. The 'Methods' screen (Figure 2) displays all of the methods that are installed in the Maxprep® software. Methods are shown either as single line entries or with a folder icon if the administrator has defined lab-specific variant versions of the Promega-supplied methods. Select the folder icon for the QuantiFluor® Dye Quantitation method to expand the list of variant methods that were created.

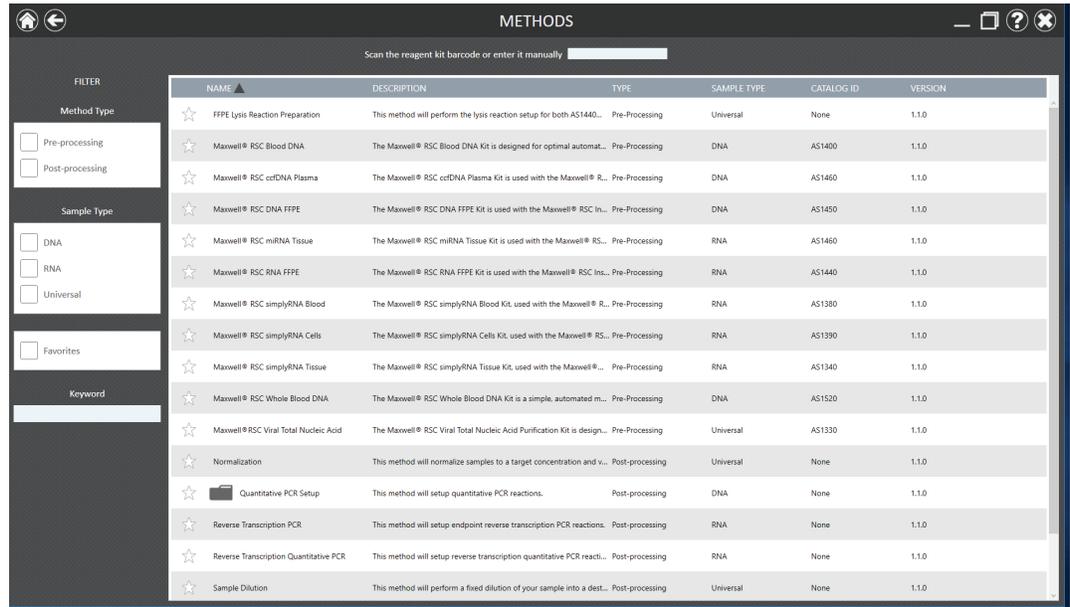


Figure 2. 'Methods' screen. The 'Methods' screen displays the methods installed in the Maxprep® software. Select the method to run, and then press the **Proceed** button.

4. Use one of the following two mechanisms to select a QuantiFluor® Dye Quantitation method:
 - a. Select the method or variant from the list of installed methods.
 - b. Use the Filter check boxes on the left side of the screen to display only post-processing methods, display only methods marked as Favorites or perform a keyword search for a specific word or phrase in any of the descriptive fields for the method. After filtering the list, select the method or variant from the list of displayed methods.

Regardless of the mechanism used to select a method, the selected method is highlighted and will display a **Proceed** button on the right side of the entry. Press the **Proceed** button to open the Method Run Environment (Figure 3).

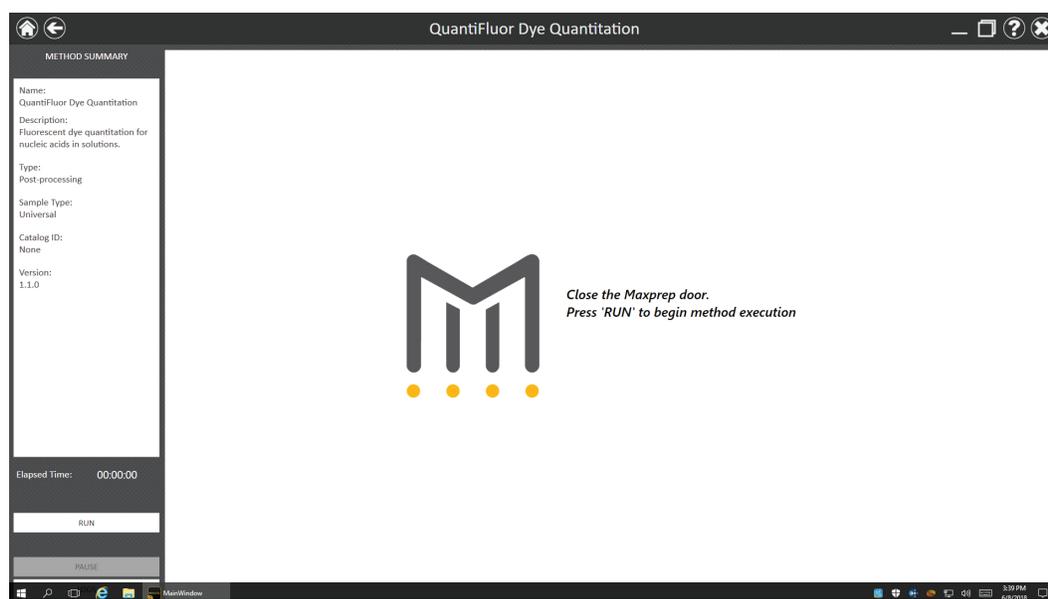


Figure 3. Method Run Environment screen. The Method Run Environment screen displays a summary of the selected method on the left side of the screen. Close the door of the instrument and select the **Run** button to begin method execution.

2.4 Starting a Maxprep® Method

The Method Run Environment displays the name of the selected method in the title bar and a summary of the information describing the selected method on the left side of the screen. If an incorrect method was chosen, select the **Home** or **Back** button at the top left corner of the screen to return to the 'Home' screen or return to the 'Methods' screen, respectively.

To start method execution, close the door of the instrument and select the **Run** button in the lower left corner of the screen. Once method execution is started, the timer under the *Elapsed Time*: header will start, and the main portion of the screen will indicate that the system is initializing.

2.5 Entering Run-Specific Information

After the system has initialized, a user interface is displayed in the main portion of the Method Run Environment. The user interface requests information necessary to process this method.

The first screen requests information regarding general method run parameters such as: Input Labware, Destination Labware and whether to perform a 100-fold dilution of samples prior to quantitation (Figure 4).

Note: When creating variant quantitation methods, the administrator defines the parameters specific to the quantitation being performed. These parameters cannot be modified at run time. See Section 4 for information on creating a quantitation variant method.

Input Labware
Maxwell RSC 48 Trays
Samples are in elution tubes

Maximum Input Samples 48

Destination Labware
Costar Flat Black Plate
Part number 3915

Run parameters are defined in method variants. These parameters include reaction components, standard curve settings, and controls usage. Click below to view the settings for this run.

Dilute Samples 100-Fold

Variant Information

PREV NEXT

Figure 4. General Run Parameters screen. The General Run Parameters screen requests information about input labware, destination labware and sample dilution. Select the **Variant Information** button to view the administrator-defined settings for this quantitation method.



Note: When “Dilute Samples 100-fold” is checked, Samples and Controls will be diluted 100-fold prior to quantitation setup.

1. Use the drop-down menus to specify the Input Labware and Destination Labware to be used for this run. Samples are placed on the system in the Input Labware, and the quantitation reaction is prepared in the Destination Labware. Maxwell® Deck Trays, plates and tubes can all be used as input labware options for QuantiFluor® dye quantitation.
2. If desired, check the box to “Dilute Samples 100-fold” prior to quantitation. Samples that are expected to have a high concentration may require a 100-fold dilution prior to quantitation so that they fall within the range of the assay.

3. At the top right corner of the screen is an indication of the Maximum Number of Samples allowable within this quantitation run. The number displayed is based on:
 - The number of sample positions in the input labware
 - The number of controls specified
 - The number of standards specified
 - The number of standard curve and sample replicates
 - The available space in the destination labware
4. To view the administrator-defined settings for this quantitation variant, press the **Variant Information** button (Figure 5).
5. After entering values for Input Labware and Destination Labware, select the **Next** button.

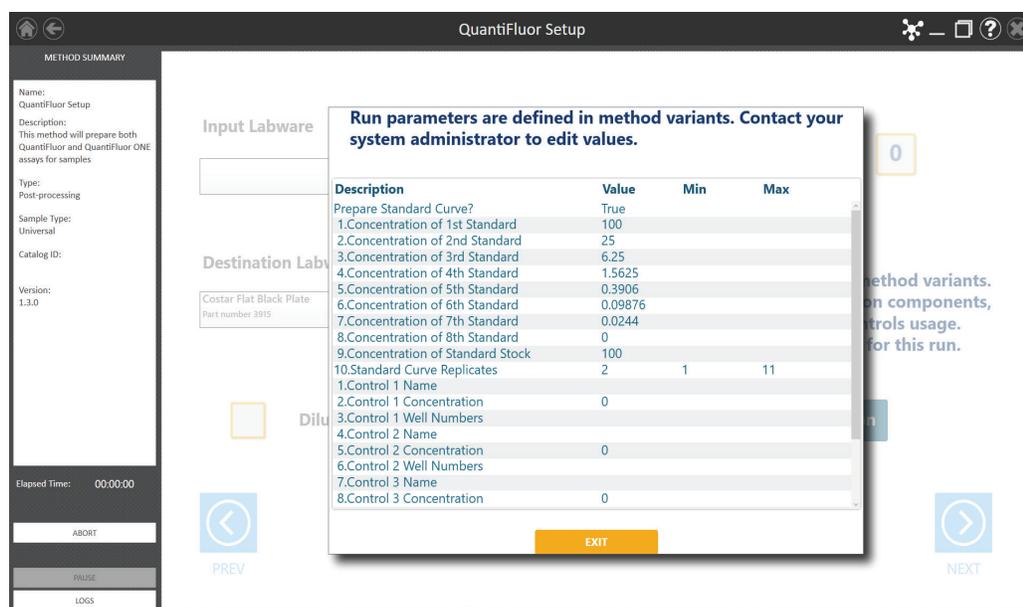


Figure 5. Viewing Quantitation Setup information. Press the **Variant Information** button on the interface to view the administrator-defined quantitation setup details for this variant method.

After selecting **Next**, the user interface requests information regarding the samples present in the input labware. The nature of the screen will depend on the type of input labware chosen and whether Portal is active.

When connected to the Portal software, a bar code is requested for each deck tray or plate to be processed (Figure 6). The screens in Figure 6 are only displayed when the Maxprep® software is connected to the Portal software. Select the text box and scan or enter the deck tray bar code(s) for the Maxwell® deck trays or the plate bar code. After entering bar codes for the labware, press the **Query Portal** button to retrieve sample identifiers from the Portal software for the most recent activity associated with the labware. The number of sample identifiers retrieved from the Portal software is displayed on the screen. Press the **Exit** button to display any records retrieved from the Portal software query or to proceed without querying the Portal software and manually enter sample identifiers.



Note: When not connected to the Portal software, the screens shown in Figure 6 are not displayed.

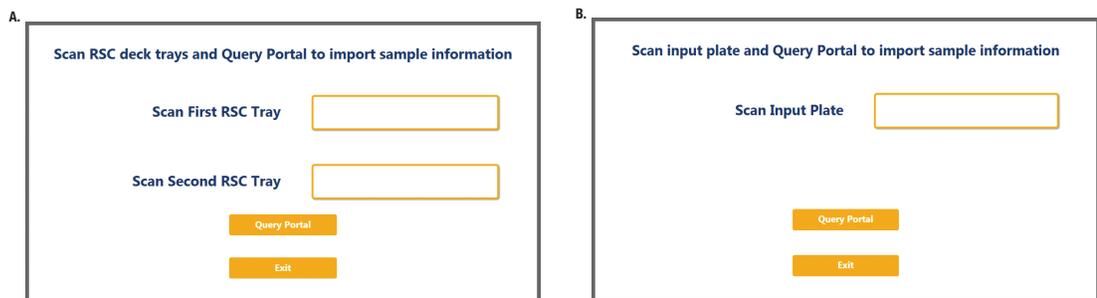


Figure 6. Querying Portal for sample bar codes. These screens are displayed only when the software is connected to the Portal software. Press the **Query Portal** button to search the Portal software for sample identifiers associated with the deck tray or plate bar code(s). Press **Exit** to display the sample identifiers retrieved from Portal or to proceed without querying Portal. **Panel A.** Scan or enter bar code information for Maxwell® Deck Tray(s) to be processed.

Panel B. Scan or enter bar code information for the plate to be processed.



Note: The designation RSC on this screen generically refers to any supported 16-position or 24-position Maxwell® instrument deck tray.

After pressing the **Exit** button, or when not connected to the Portal software, the user interface will display a representation of the labware. If applicable, any sample identifiers returned from the Portal software query are populated on the labware display and shown as black cartridge or well positions. Labware positions with no sample identifiers are displayed as gray cartridge or well positions. To enter or modify sample identifiers, select the desired labware position, then select the text box on the right side of the screen (Figure 7). Scan or enter the sample identifier information for the position. When entering sample identifiers, press the **Add Sample** button to accept the entry or press **Enter** on the keyboard to accept the information and move to the next position.

If more samples are present on the input labware than can be processed with this variant, a message will be displayed at the top of the interface screen indicating the number of samples that will have to be removed before proceeding. To remove samples from processing, select a position, Ctrl-click to select multiple positions, or click and drag across several consecutive positions to select them. After selecting positions, press the **Remove Samples** button to remove them from being processed during this run.

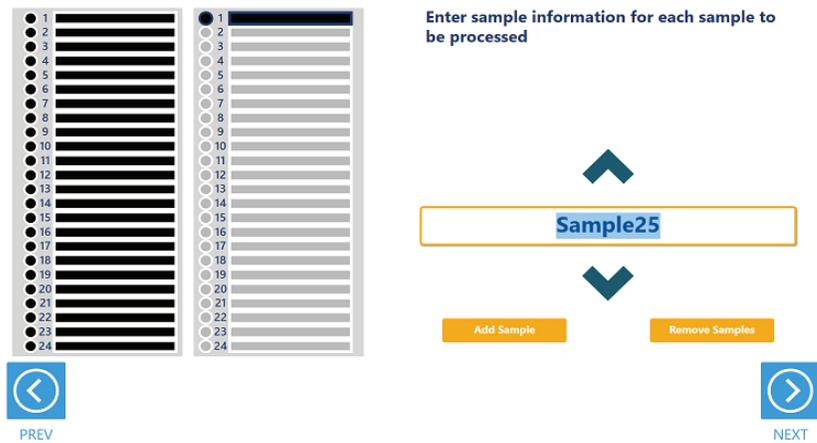


Note: When not connected to the Portal software, the **Requery Portal** button is not displayed.

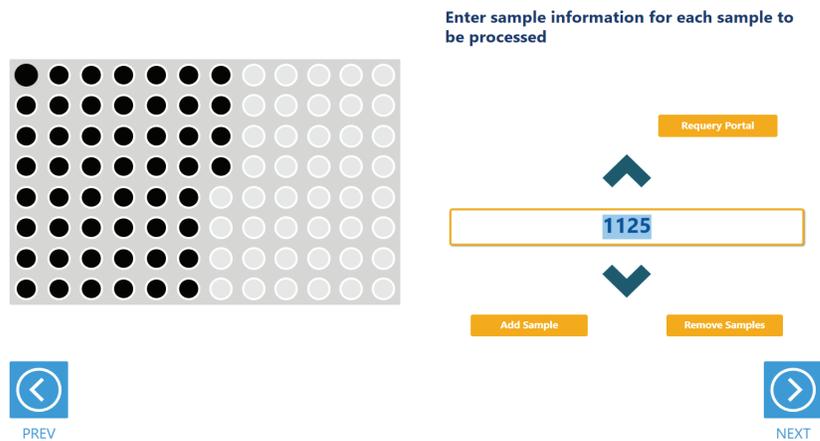
If sample identifiers are removed or edited incorrectly, it is possible to reset the labware to the original information retrieved from Portal. Press the **Requery Portal** button to repeat the process of retrieving sample identifiers from the Portal software for a piece of labware based on the labware bar code.

Once satisfied with the labware positions and sample identifiers to be processed in this quantitation setup, press the **Next** button to proceed to a display of the quantitation plate layout.

A.



B.



14881CA

Figure 7. Entering sample identifiers. Input labware is displayed on the interface and allows entry of sample identifier information. Any labware positions that have sample identifier information (either retrieved from Portal software or manually entered) are displayed in black. To enter sample identifiers, select the labware position and then select the text box on the right side of the screen. Scan or enter sample identifiers and press the **Add Sample** button to accept the sample identifier or press **Enter** on the keyboard to accept the sample identifier and move to the next position. **Panel A.** Interface screen for Maxwell® deck trays as input labware. **Panel B.** Interface screen for a plate as input labware.

When selecting tubes as an input labware type, the interface will prompt the user to scan bar codes on three tube racks at Tracks 22–24 (Figure 8). Scanning tube racks will determine the type of tube rack being used, the types of tubes defined for that rack type and the sample identifiers for each tube.

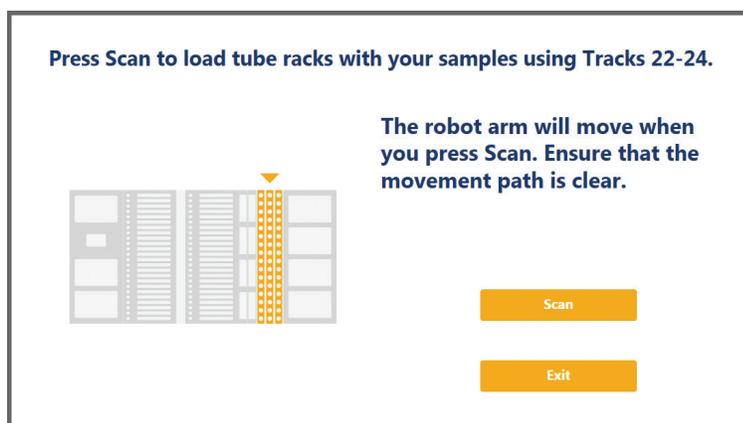


Figure 8. Scanning tube racks. Tube racks at Tracks 22–24 must be scanned when tubes are selected as an input labware type. Scanning tube racks will determine the type of tube rack being used, the types of tubes defined for the rack type and the sample identifiers for each tube.

To scan sample tube racks:

1. Remove tube racks from the system. Load tubes into the tube racks with bar codes visible in the windows on the right side of the carrier.
2. Load the tube racks on the instrument to identify the type of tube rack being used and scan bar codes on the individual tubes.
 - a. Press the **Scan** button.
 - b. The instrument arm will slowly move to the position for scanning the first primary sample tube rack.
 - c. With the tube rack removed from the system, the green loading light where the tube rack should be loaded will flash.
 - d. Place the back edge of the tube rack on the loading platform so that the rearmost bar code on the tube rack (rack identifying bar code) is just in front of the bar code scanner.
 - e. Slide the tube rack into the system until it fully contacts the back of the instrument. During this time the bar code scanner will flash each time it reads a tube position.

Note: Do not remove or insert tube racks while the instrument arm is moving. After scanning a tube rack, wait until the instrument arm has moved to the next position before inserting a tube rack.

- f. When the tube rack is fully seated in the instrument, the loading light at the front of the instrument will stop flashing. Approximately five seconds after the tube rack is loaded into the instrument, the instrument arm will slowly move to the right for loading of the next position.
- g. Repeat steps c–f for all three tube racks. If sample tubes are not present in all three carriers, press **Stop** when the instrument arm has moved after scanning the last carrier containing tubes.

- Once all tube racks are scanned, the interface will display the number of samples that were successfully scanned. Press the **Exit** button to proceed. If multiple tube labware definitions exist for a given rack, a popup screen is displayed to select which tube type is being used on that rack (Figure 9). Select the tube type being used for each rack from the drop-down menus. All tubes on an individual tube rack must be of the same type.

The screenshot shows a dialog box with the following content:

- Multiple tube types possible for the scanned tube racks.**
Select the correct labware for each tube rack.
- Rack 1: Track Position 22**
A drop-down menu showing "2.0 ml Screw Cap Tubes" and "Sarstedt Tubes".
- Rack 2: Track Position 23**
A drop-down menu showing "2.0 ml Screw Cap Tubes" and "Sarstedt Tubes".
- Rack 3: Track Position 24**
A drop-down menu showing "2.0 ml Screw Cap Tubes" and "Sarstedt Tubes".
- An orange **Exit** button at the bottom.

Figure 9. Choosing a tube type. When multiple tube types are defined for a tube rack, it is necessary to select which tube type is being used on any given rack. Each tube rack can use a different tube type, but all tubes within a single rack must be of the same type.

The next user interface screen displays the three tube racks with a green indicator at each position where bar code information was scanned (Figure 10). Select a tube position and confirm the bar codes for each tube using the **Up** and **Down** arrow keys to navigate through tube positions. If no bar codes are present on the tubes, it is still required to enter sample identifier information for each position to be processed. Select the tube position and enter sample identifier information. Press **Enter** on the keyboard to accept the sample identifier and advance to the next tube position.

If more samples are present on the input labware than can be processed with this variant, a message will be displayed at the top of the interface screen indicating the number of excess samples that will have to be removed before proceeding. To remove samples from processing, select a tube position and press **Delete** on the keyboard. Use the **Up** and **Down** arrow keys on the keyboard to navigate through tube positions to delete additional sample identifiers. To rescan tube bar codes, change tube racks or specify a different tube type, press the **Rescan Tubes** button. This will reopen the screen used to scan tube racks (Figure 8).

Once satisfied with the labware positions and sample identifiers to be processed in this quantitation setup, press the **Next** button to proceed to a display of the quantitation plate map.

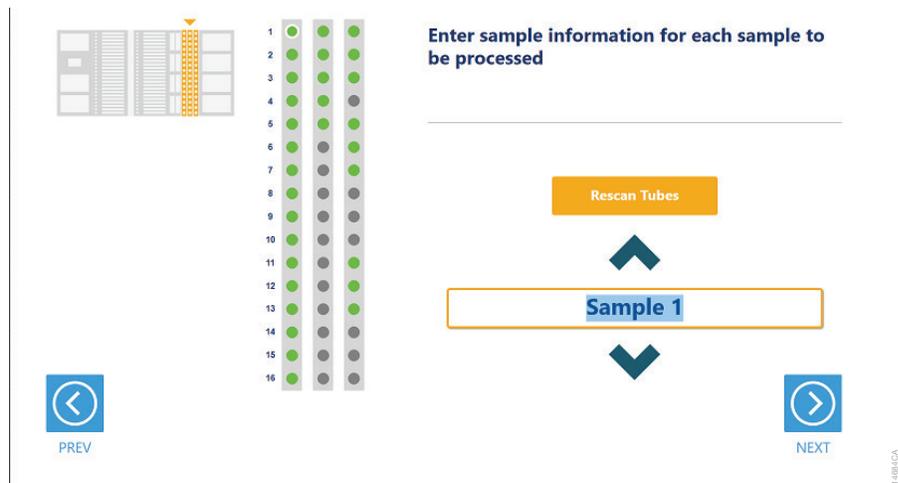


Figure 10. Tube bar code confirmation/entry. Tube positions for which bar code information was scanned are indicated in green. All samples to be processed must have sample identifier information. If no bar codes are being used, manually enter a sample identifier for each position to be processed by selecting the position and entering an identifier.

After selecting the labware positions used for the quantitation setup, the user interface presents a screen that displays the quantitation plate map (Figure 11). This is an informational screen only and cannot be modified. The locations of samples, controls and standards are indicated on the plate with samples displayed in black, controls in yellow and standards in blue. Select any well position on the plate map to view the identifying information for that well. After reviewing the plate map, press the **Next** button to begin instrument setup or press the **Back** button to return to the beginning of the quantitation setup process.

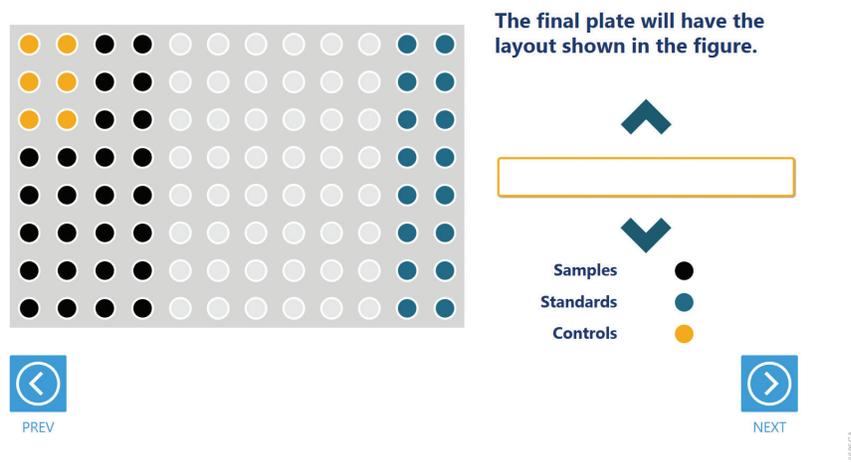


Figure 11. Quantitation Plate Map. A Quantitation Plate Map is displayed to show the positions of samples, controls and standards. Samples are shown as black wells, controls as yellow wells and standards as blue wells. Select any well position to view identifying information for that well.

2.6 Instrument Setup Instructions

Instrument setup proceeds from left to right on the Maxprep® Liquid Handler. The loading platform of the Maxprep® Liquid Handler uses a track system to indicate where carriers are loaded on the system. In these instructions each carrier referenced will list the starting and ending track number that are spanned by the carrier. Make sure that carriers are placed correctly on the instrument at each step of the user interface. Screens are presented to indicate the types of labware that should be placed on the instrument, volumes of reagents that should be provided and allow entry of reagent bar code information.

All deck setup screens display a miniature view of the entire instrument in the upper left corner of the screen with the current position highlighted (Figure 12). Below this image is a summary of the selected run parameters. On the right side of the screen is a depiction of the carrier and instructions for what should be present at each position on the carrier. For each position where labware or reagents must be placed there is a check box. As labware is loaded at each position, check the box to indicate that the instruction was performed. It is not possible to proceed to the next instrument setup screen until a check is present in all check boxes on the screen.

2.6.1 Utility Carrier (Tracks 1–6)

The Utility Carrier is a fixed position and cannot be removed from the instrument. It contains a Heater/Shaker unit at Site 1 (back of the instrument), instrument tools (gripper paddles, plunger tools) at Site 2 and two labware positions at Site 3 and Site 4 (front of the instrument; Figure 12).



Note: The 1.1ml Square Well Plate at Site 3 is only shown if the option to dilute samples 100-fold was selected at the beginning of the QuantiFluor® Dye Quantitation method.

Follow the information on the right side of the screen regarding placement of labware. For instance, on the screen in Figure 12, there should be no labware present on the Heater Shaker device at Site 1, the Gripper Paddles and Plunger Tools are present at Site 2, a 1.1ml Square Well Plate is present at Site 3 and a Reaction Plate is present at Site 4.

For the Reaction Plate at Site 4, selecting the **Enter Plate Details** button or the check box at Site 4 displays details and allows entry of bar code information for the Reaction Plate (Figure 13). Place the indicated Reaction Plate labware on Site 4. By default the bar code for the Reaction Plate is set to the date and time the method was started. Select the "Labware Barcode" text box to scan or enter a different bar code for the Reaction Plate. If Portal sample tracking is enabled, this Labware Barcode can be used to import the reaction plate map into a GloMax® Discover or Explorer multimode microplate reader.

After placing labware, check the boxes next to each position to activate the **Next** button at the bottom right corner of the screen. Press the **Next** button to proceed to continue instrument setup.

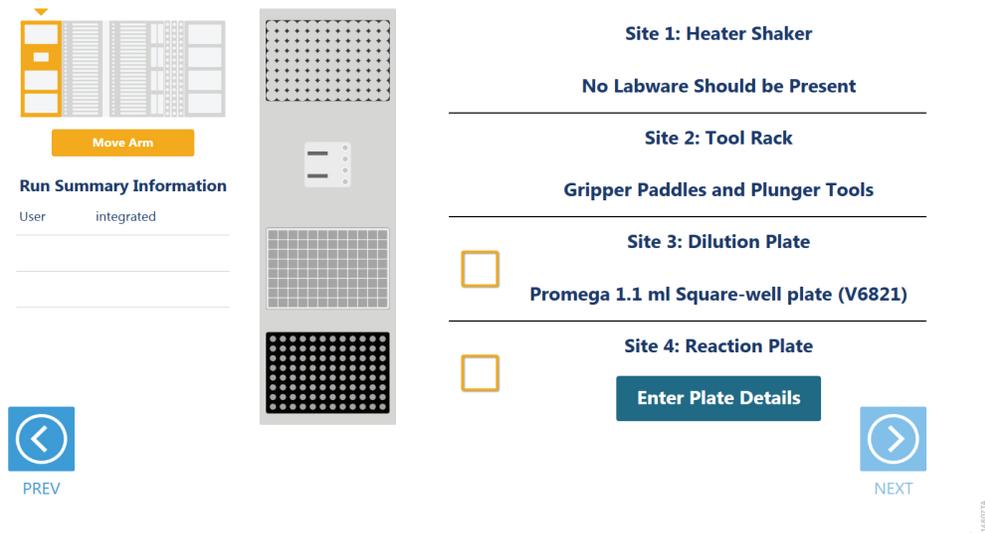


Figure 12. Deck Setup screen for the Utility Carrier. This carrier cannot be removed from the system. It contains a Heater/Shaker for incubation of samples, tools and two labware positions. Place the indicated labware on the carrier, and check the boxes to activate the **Next** button. Press **Next** to continue instrument setup.

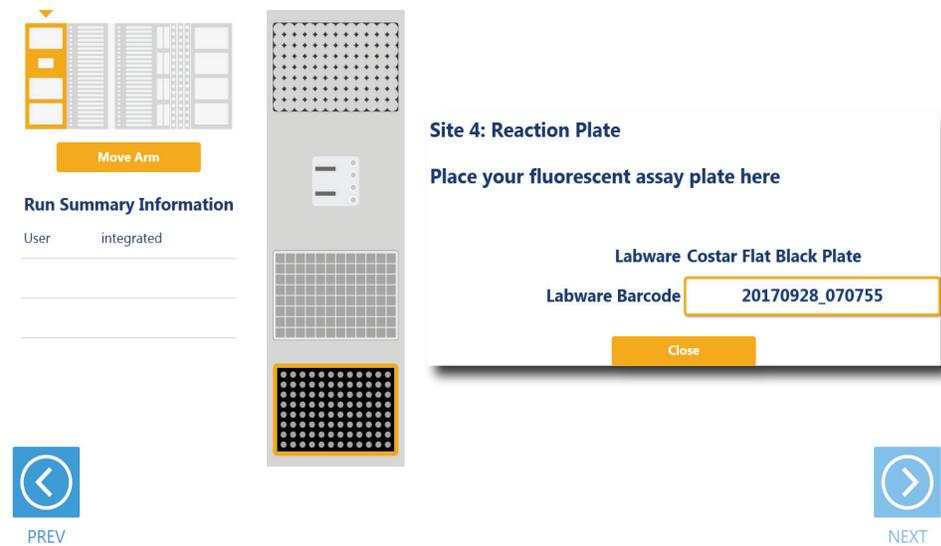


Figure 13. Reaction Plate Details screen. For the Reaction Plate at Site 4, press the **Enter Plate Details** button to display information about the plate. Select the “Labware Barcode” text box to scan or enter a bar code for the Reaction Plate. The default bar code for the plate is the date and time the quantitation method was started.

2.6.2 Maxwell® Deck Tray Carriers (Tracks 7–12, 14–19)

The Maxwell® Deck Tray Carriers are used to place either Maxwell® deck trays or a plate on the instrument as input labware. Instructions for both input labware types are shown below.

Notes:

- a. Refer to the miniature instrument image on the left side of the screen to see which position the displayed Maxwell® Deck Tray Carriers should occupy on the instrument.
- b. The Maxwell® Deck Tray Carriers are not intended to be directly next to each other on the instrument. A Reagent Carrier should be placed or an empty track should be left at Track 13 between the Maxwell® Deck Tray Carriers to make sure they are loaded correctly.

Maxwell® Deck Trays as Input Labware

There are three types of Maxwell® Deck Tray Carriers: one for 16-position Maxwell® deck trays, one for 24-position Maxwell® Front Deck Trays and one for 24-position Maxwell® Back Deck Trays. The choice of which deck tray type will be used for the run is made when the input labware is selected for the run. These carriers can be removed from the system and replaced depending on the type of deck trays that are being used for this run. The quantitation methods use eluates in the Maxwell® elution tube position as samples for the run. In the center of the screen (Figure 14) is an image of the deck trays where the positions to be processed are indicated as black cartridges. If only one deck tray is to be processed, the other deck tray will be shown as a gray rectangle with no cartridges present. Elution tubes should be securely loaded with the caps open on the deck trays at the indicated positions. Place Maxwell® deck trays, minimally containing the specified number of elution tubes placed at the elution positions, on the Maxwell® Deck Tray Carriers. Shown on the right side of the screen are the bar codes for each Maxwell® deck tray. The first or Front deck tray is placed on the left carrier at Tracks 7–12, and the second or Back deck tray is placed on the right carrier at Tracks 14–19.

Check the boxes next to the deck tray bar codes as you load the Maxwell® deck trays on the Maxprep® Liquid Handler. Once the boxes are checked, the **Next** button in the bottom right corner of the screen will become active. Press the **Next** button to move to the next instrument setup screen.

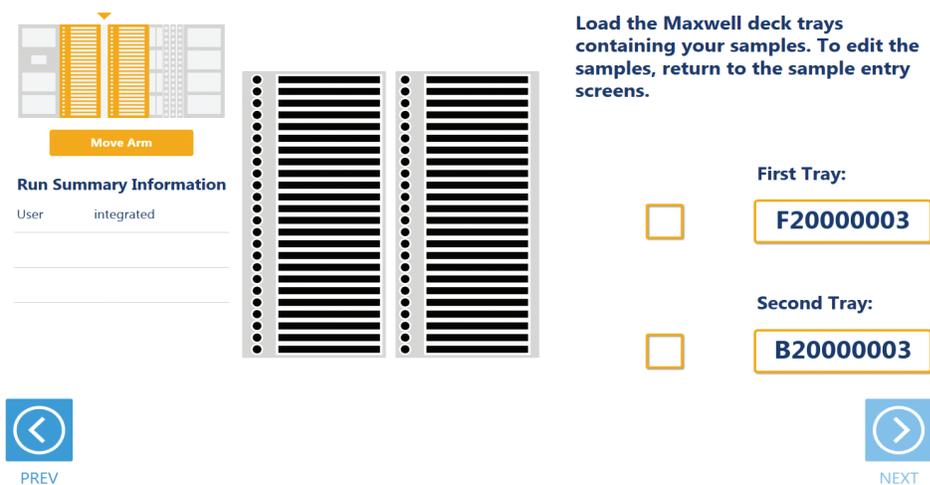


Figure 14. Maxwell® Deck Tray Carriers. These carriers are used to hold 16-position Maxwell® deck trays or 24-position Maxwell® deck trays (Front and Back). Place Maxwell® deck trays with elution tubes at the indicated positions on the deck tray carriers as instructed.

Plate as Input Labware

When a plate is selected as an input labware type, the front-most plate position on the left Maxwell® Deck Tray Carrier is used (Figure 15). Any type of Maxwell® RSC Deck Tray Carriers can be used for positioning the sample plate. Remove the Maxwell® deck tray (if present) and the elution magnet from the left Maxwell® Deck Tray Carrier. Place the sample plate on the left Maxwell® Deck Tray Carrier as indicated. The bar code of the sample plate is shown on the right side of the screen. After placing the sample plate, check the box to activate the **Next** button. Press the **Next** button to move to the next instrument setup screen.

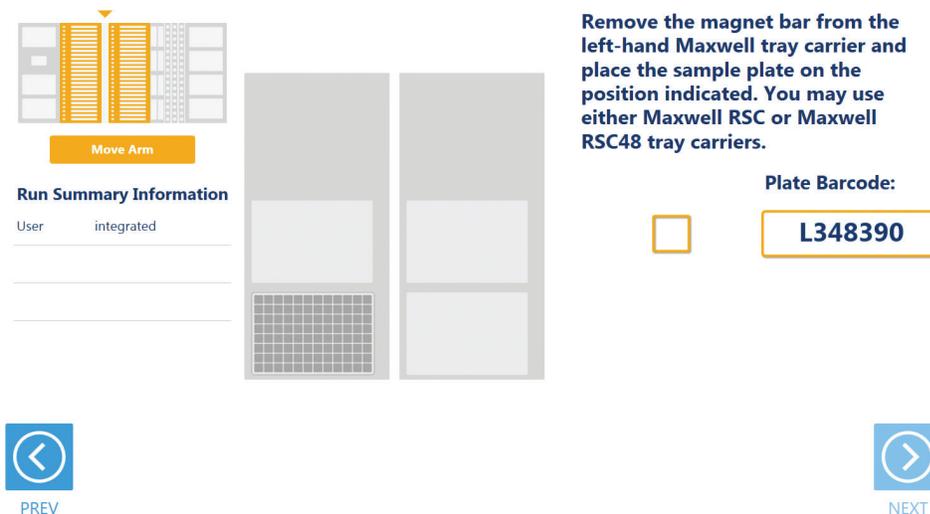


Figure 15. Sample Plate Position. When using a plate as input labware, the plate is placed on the front plate position of the left Maxwell® Deck Tray Carrier as indicated. Any type of Maxwell® Deck Tray Carrier can be used. Remove any Maxwell® deck tray and the elution magnet from the Maxwell® Deck Tray Carrier before placing the sample plate.

The Reagent Carrier screens display the reagents that should be placed at each site on a reagent carrier (Figure 16). Reagents are placed in either a Maxprep® 3-Position Reagent Tube Holder or a Maxprep® Reagent Reservoir, 60ml, as indicated on each screen.

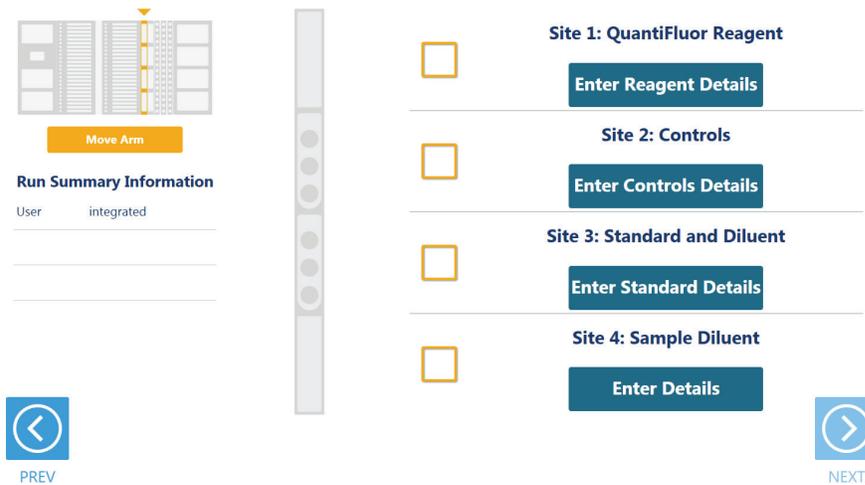


Figure 16. Reagent Carrier Screens. The name of the reagent to be placed at each carrier site is indicated. Press the **Details** button for any individual site to see details and enter reagent lot information.

Press the **Details** button to see details specific to the reagent(s) that should be placed at each site on the reagent carrier (Figure 16). The details are specific to the type of reagent placed at a given site. When finished viewing instructions for a reagent site, press **Next Site** to move to the next occupied site on the reagent carrier or press **Close** to leave the reagent details and return to the main reagent carrier screen. The act of viewing reagent details will cause the check box for the reagent to be checked. All check boxes must be checked to activate the **Next** button on the main reagent carrier screen. Press **Next** to move to the next instrument setup screen.

For tube-based reagents on the first reagent carrier, place a Maxprep® 3-Position Reagent Tube Holder (Cat.# AS9409) at the site and add individual tubes containing the reagent specified at the indicated positions in the tube holder. If there is not sufficient reagent present in a tube to complete the run, a warning prompt will be shown to place a new tube of reagent.

When reagents are to be placed at a reagent site in a trough, transfer at least the indicated volume of reagent into a Maxprep® Reagent Reservoir, 60ml, and place it at the designated site on the carrier. If there is not sufficient reagent present in the trough to complete the run, a warning prompt will be shown to add more of the specified reagent to the trough position for this reagent.

First Reagent Carrier

Reagent Type	Labware	Details
QuantiFluor® Reagent	Maxprep® Reagent Reservoir, 60ml, containing prepared QuantiFluor® Reagent	Add at least the minimum volume of prepared QuantiFluor® reagent indicated to the Maxprep® Reagent Reservoir, 60ml, at this site. Enter bar code for the QuantiFluor® reagent as desired. Note: For QuantiFluor® dsDNA, QuantiFluor® RNA and QuantiFluor® ssDNA Systems, dilute the QuantiFluor® dye in TE as directed in the chemistry manual before adding it to the reagent reservoir. QuantiFluor® ONE dsDNA reagent can be added to the reservoir directly.
Controls	1.5ml or 2.0ml tubes containing controls	The names of the controls are shown. Place controls in open tubes from back to front in the Maxprep® 3-Position Tube Holder as indicated. Enter bar codes for each control as desired.
Standard and Diluent	1.5ml or 2.0ml tubes containing standard and standard diluent	Place the open standard tube in the rearmost position (Position 1) and the open diluent tube in the middle position (Position 2) of the Maxprep® 3-Position Tube Holder. The minimum required volume of standard diluent is indicated. Enter bar codes for each reagent as desired. Note: The Maxprep® QuantiFluor® Dye Quantitation method was verified using the standards provided in the QuantiFluor® Assay kits. The user should validate the suitability of alternative nucleic acid sources for use as the assay standard.
Sample Diluent	Maxprep® Reagent Reservoir, 60ml, containing sample diluent	Add at least the minimum volume of sample diluent indicated to the Maxprep® Reagent Reservoir, 60ml, at this site. Enter bar code for the sample diluent as desired. This position is only prompted if samples will be diluted 100-fold prior to quantitation.

2.6.3 Sample Tube Carriers (Tracks 22, 23, 24)

When tubes are selected as the input labware, the tube carriers at Tracks 22–24 will be displayed (Figure 17). Scanning or entry of sample tube bar codes occurred earlier in the quantitation method, and the tube racks should already be present at these three track positions. Confirm that tubes are present at the positions indicated in green on each of the tube racks displayed. All tubes within each tube carrier should be of the same type. Check the box to activate the **Next** button. Press the **Next** button to proceed to the next instrument setup screen.

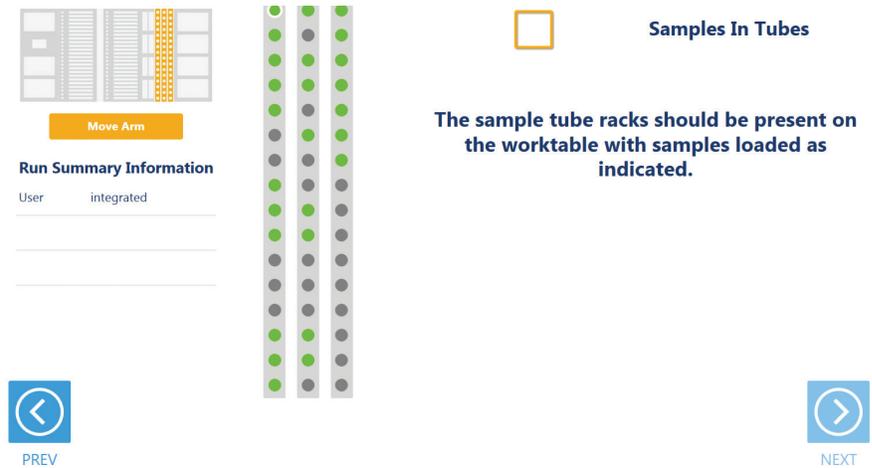


Figure 17. Sample Tube Rack screen. When tubes are the input labware, the three sample tube racks are displayed. Confirm that tubes are present at each position indicated in green and that all tubes within a rack are of the same type.

2.6.4 Tip and Plate Carrier (Tracks 25–30)

Tips and standard curve labware are placed on the tip and plate carrier (Figure 18). Site 1 on this carrier is a plate position that is used to prepare the standard curve serial dilutions. The labware used at this position can be either an amplification plate or strip tubes placed in the first column of an Amplification Plate Base (Figure 19).

Note: Strip tubes, unskirted and partially skirted amplification plates should be seated in an Amplification Plate Base before being placed on the system. Fully skirted amplification plates can be placed directly on the system.

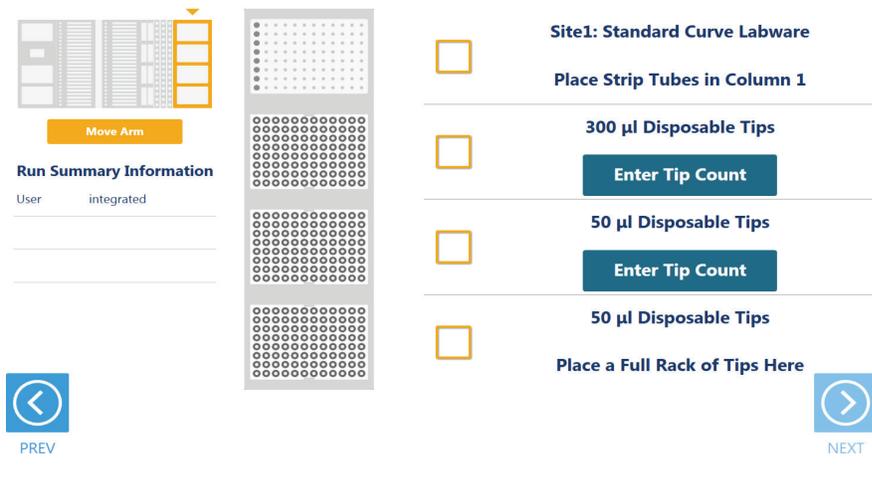


Figure 18. Tip and Plate Carrier screen. Place the specified labware at each site. Site 1 contains either an amplification plate or strip tubes in the first column of an amplification plate base. Sites containing a tip rack indicate the type of tips that should be placed there and whether a partial or full rack of tips needs to be placed. For partial tip racks, press the **Enter Tip Count** button to specify the first available tip in the partial tip rack

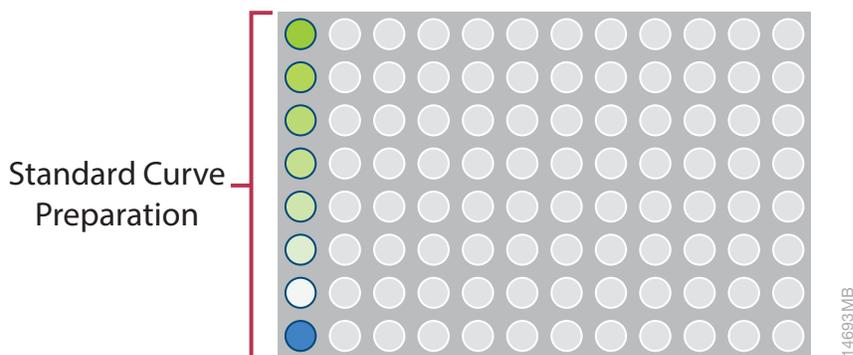


Figure 19. Standard Curve Labware. The standard curve labware is used to prepare standard curve serial dilutions. Place a PCR plate or strip tube in the first column of an amplification plate base at Site 1 of the Tip and Plate Carrier (shown in Figure 18).

Sites 2–4 on this carrier are used to hold tip racks. Place tips of the specified type at each site on the carrier. Sites at which either a full or partially filled tip rack can be placed will have an **Enter Tip Count** button. Press the **Enter Tip Count** button and select the first available tip present in the tip rack (Figure 20). Entering a tip count and pressing the **Close** button will act to check the check box for the specified site. If a tip count is not entered, the software will assume a full rack of tips is located at the specified position. For all other sites, once you have added the requested labware, check the check box next to the site. Only after all check boxes on the screen are checked will the **Next** button become active. Press the **Next** button to move to the next screen.

Note: Confirm that the appropriate tips are placed at each tip position before starting the method. Tip types used at each position differ between preprocessing and post-processing methods.

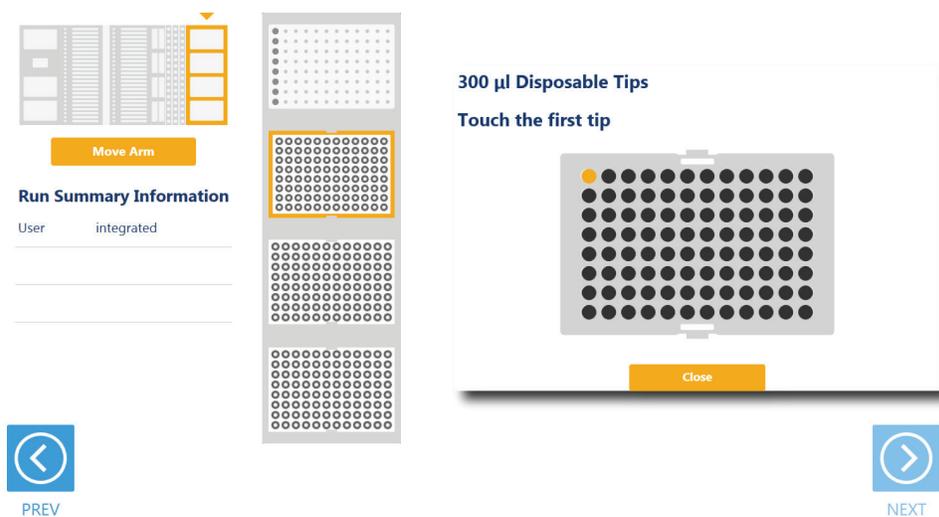


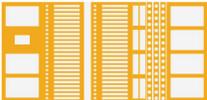
Figure 20. Selecting the first tip in a partial tip rack. For sites at which a partial or full tip rack can be placed, press **Enter Tip Count** to specify the first tip present (in gold) in the partial tip rack. Press **Close** to return to the display of all sites on the carrier. Check all check boxes after loading labware to proceed.

The final screen of the instrument setup presents a list of items that should be checked before starting the run. Confirm that:

- All carriers are completely seated in the instrument
- All labware are loaded at the correct locations
- Tip waste bin is empty if necessary
- The door of the instrument is closed

Once the items in the list are checked and the door of the instrument is closed, press **Next** to start automated sample processing (Figure 21). This is the last chance to return to any of the instrument setup screens and confirm instrument setup before sample processing will begin.

During the run, if there is a need to pause instrument operation in order to resolve any unforeseen issues, press the **Pause** button in the lower left corner of the screen. If it is necessary to abort the run for any reason, press the **Abort** button in the lower left corner of the screen.



Setup Complete

- ◆ Ensure that all carriers are completely pushed back.
- ◆ Verify that all reagents and labware are loaded at the correct locations.
- ◆ Empty the waste bin, if necessary.
- ◆ Close the door.
- ◆ Press 'NEXT' to begin processing.





PREV



NEXT

Run Summary Information	
User	integrated
Sample Number	75
Sample Replicates	1

Figure 21. Final instrument setup screen. This screen presents a list of items that should be checked before starting automated processing. It also provides a final chance to return to any of the previous instrument setup screens before automated processing begins. To start processing, close the instrument door and press the **Next** button.

2.7 Maxprep® Liquid Handler QuantiFluor® Dye Quantitation Protocol

The Maxprep® Liquid Handler will prepare quantitation reactions as indicated by the variant selected. The following steps are performed by the Maxprep® Liquid Handler:

1. The system checks that there is sufficient volume in the reaction mix trough to process all samples, controls and standards.
2. **Optional:** The system checks that there is sufficient volume in the sample diluent trough to dilute samples and controls.
3. **Optional:** Transfer of sample diluent from the sample diluent trough to the dilution plate.
4. Transfer of standard diluent to the standard curve labware based on the concentrations of standards specified in the variant method.
5. Serial dilution of standards in the standard curve labware based on the concentrations of standards specified in the variant method.
6. **Optional:** Dilution of samples 100-fold in the dilution plate.
7. **Optional:** Dilution of controls 100-fold in the dilution plate.
8. Transfer of QuantiFluor® Reagent to the reaction plate.
9. Transfer of samples to the reaction plate based on the sample assay volume specified in the variant method.
10. Transfer of controls to the reaction plate based on the sample assay volume specified in the variant method.
11. Transfer of standards to the reaction plate based on the sample assay volume specified in the variant method.
12. Method is complete. Open instrument door, remove the reaction plate and read in a fluorescence plate reader. Remove primary samples, plates and used tips from the waste bin and discard as hazardous waste following your institution's recommended guidelines. Either discard or tightly cap and store remaining reagents.



Consumables for Maxprep® methods are designed to be used with potentially infectious substances. Use appropriate protective equipment (e.g., gloves and goggles) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances when used with this system.

2.8 Analysis of Results

Because the QuantiFluor® Dye Quantitation method transfers the same volumes of samples, controls and standards to the quantitation reaction plate, it is possible to directly correlate sample concentrations to the concentrations present in the standard curve. Follow the instructions below to analyze quantitated samples.

1. After quantitation reaction setup is complete, read the resulting reaction plate on a fluorescence plate reader as directed by the appropriate QuantiFluor® Technical Manual.
2. Average the relative fluorescence values from the blank wells on the standard curve and subtract from the relative fluorescence values for standards, controls and samples.
3. Create a scatter plot of the non-blank standard curve points vs. blank-subtracted relative fluorescence units. Add a trendline to the graph using either a power or linear fit equation. We recommend using a power fit trendline.
4. Using the formula for the standard curve trendline, interpolate the values of the samples and controls based on their blank-subtracted relative fluorescence values.
5. Apply a 100-fold multiplier to the calculated sample and control concentrations if the samples and controls were diluted 100-fold as part of the quantitation variant.

The calculated concentrations provide an indication of the initial nucleic acid content of the samples and controls.

The GloMax® software can import the plate map based on the Reaction Plate ID and automatically calculate the sample concentrations from Portal. For more information, refer to *GloMax® Discover System Operating Manual #TM397* or *GloMax® Explorer System Operating Manual #TM433*. Exporting a plate map of the resultant Reaction Plate using Portal Access Software is also possible. For more information, refer to the *Portal Access Software Technical Manual #TM549*.

3

Potential Errors and Warnings

The Maxprep® Liquid Handler QuantiFluor® Dye Quantitation method is designed to provide notification and resolution options for several potential error/warning states. Below are listed several possible error/warning states that may be observed during a method. Always carefully read any warning or error messages to find out how to proceed. If in doubt about how to proceed with any error state, the safest option is to choose the Abort button to end the run.

3.1 Intrusion Sensor

The intrusion sensor on the Maxprep® Liquid Handler checks for any potential physical obstructions that could prevent gantry movement on the instrument. Prior to any instrument movement (e.g., initialization, bar code scanning, arm movement, resuming a method after a pause, performing a maintenance method), always be sure to check that all carriers on the system are fully pushed to the back of the instrument and that nothing could impede the progress of gantry movements. An LED on the left front side of the deck provides a visual indicator of the intrusion sensor state. When no physical obstruction is detected, the LED is orange, but when the LED is green, a carrier or other physical obstruction has been detected by the intrusion sensor. Make sure that all carriers are fully seated on the system and that the intrusion sensor LED is orange before proceeding with any instrument operation that will result in movement of the gantry.

If the intrusion sensor does detect a physical obstruction to the gantry movement the system will display an error message (Figure 22) when the gantry attempts to move. This error does not result in an aborted run and the run can continue if you follow the steps below.

Note: During automated bar code scanning, if the intrusion sensor detects an obstruction, the error below will not be displayed and recovery is not possible. In this case, it will be necessary to abort the run, power cycle the Maxprep® Liquid Handler, and start the method run again.

1. On the 'Channel Error Recovery' screen (Figure 22, Panel A), press the **Control Panel** button in the lower left corner to open the 'Instrument Control Panel' screen (Figure 22, Panel B).
2. On the 'Instrument Control Panel' screen, press the **Unlock Door** button. Open the door of the instrument and push all carriers back. Confirm that the intrusion sensor does not detect an obstruction by checking that the intrusion sensor LED on the left side of the instrument is orange.

3. Close the instrument door and then press the **Lock Door** button on the 'Instrument Control Panel' screen.
4. In the Initialize area of the 'Instrument Control Panel' screen, make sure the radio button for Instrument is selected and then press the **Initialize** button. The gantry of the Maxprep[®] Liquid Handler will initialize.
5. Close the 'Instrument Control Panel' screen using the **X** button in the upper right corner of the screen.
6. On the 'Channel Error Recovery' screen, in the Channel Recovery Options area, press the **Select All** button. This will check the boxes for each channel at the top of the screen.
7. Press the **Execute Recovery** button at the bottom of the 'Channel Error Recovery' screen to retry the gantry movement and continue the method run.

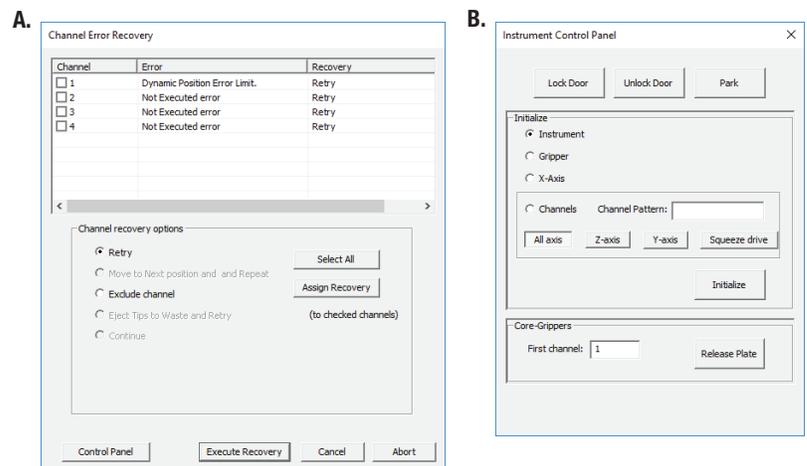


Figure 22. Error caused by a physical obstruction detected by the intrusion sensor. Panel A. When the intrusion sensor on the Maxprep[®] Liquid Handler detects a physical obstruction that would impede gantry movement prior to gantry motion, the error above is displayed. **Panel B.** Follow the instructions in the text above to reinitialize the instrument and continue processing.

3.2 Unable to Load Tips

As part of a normal method run it is necessary for the instrument to load tips on the pipetting channels. The instrument will attempt to load tips at a position twice and will automatically move to the next tip position. If the instrument runs out of tips of a given type, a warning will be displayed to replace that tip type with a full tip rack.

3.3 Insufficient Tips

While the QuantiFluor® Dye Quantitation method was developed to minimize the chance of consuming all available tips during a run, there is still a possibility that a warning message will be displayed indicating that there are no longer tips available on the tip and plate carrier (Figure 23). If this warning occurs, the instrument will pause operation and unlock the door. Open the door of the instrument, add the appropriate tip type to the first site for that tip type, close the door and press **OK** to resume processing.



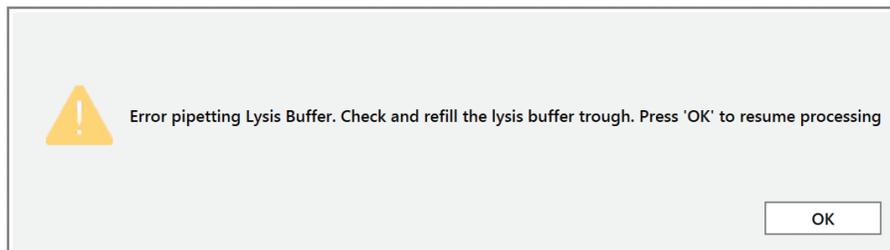
Figure 23. Insufficient tips on the system to complete a method. In the event that all tips of a given tip type are consumed during a run, the instrument will pause and unlock the door. Open the door, load more tips of the indicated type on the tip and plate carrier, close the door and press **OK** to resume processing.

3.4 Insufficient Sample

Because of the low volumes of sample used for quantitation setup, the system does not check that sufficient sample is present in sample, control or standard tubes. Make sure to provide sufficient volume of samples, controls and standards to prepare the quantitation reaction based on the sample volume indicated in the variant method.

3.5 Insufficient Liquid

If the system detects an insufficient volume of reagent in the reagent troughs or the reagent tubes to transfer the volume of reagent specified for the run, a warning is displayed (Figure 24), the system will pause and the doors will unlock. Open the instrument door and ensure that sufficient reagent is present at the position in question by either adding more reagent to a trough or the first tube of that reagent type. Make sure the door of the instrument is closed before pressing the **OK** button.

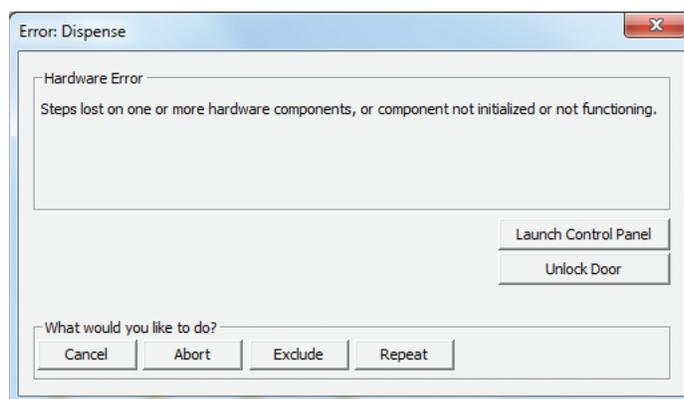


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Figure 24. Insufficient reagent on the system to complete a method. In the event that insufficient reagent is detected in a trough or tube position, a warning will be displayed; the instrument will pause and unlock the door. Open the door, replenish the indicated reagent, close the door and press **OK** to resume processing.

3.6 Hardware Crash

In the course of a method run, if the instrument experiences a physical crash of the pipetting channels into an object, an error message is displayed (Figure 25). This error message will indicate that there was a Hardware Error and the instrument has lost steps. For any situation where a physical crash of the instrument has occurred, press the **Abort** button to end the method run. Follow the instructions below for an aborted run.



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Figure 25. Hardware Crash. In the event that there is a physical crash of hardware components that results in an error message, press the **Abort** button on the error screen to end the method run.

3.7 Aborted Run

Once the QuantiFluor® Dye Quantitation method has started, aborting a run should be considered a last resort. However, there are situations where manually aborting a run (by pressing the **Abort** button in the lower left corner of the screen) may be necessary. Alternatively, there may be conditions where an instrument error could cause a run to be aborted. In the case of an aborted run you will see a warning message (Figure 26) indicating that the instrument should be powered off. If the instrument has experienced a physical crash or there are plungers, tools, or tips loaded on the pipetting channels, after powering the instrument off, resolve the crash state by following the instructions regarding recovering from an instrument crash found in the *Maxprep® Liquid Handler Technical Manual #TM509*. Power the instrument back on and restart the Maxprep® software. After any instrument crash, if there is not visible damage to the pipetting channels, it is recommended to run the Weekly Maintenance method that will check instrument calibration. If the Weekly Maintenance method produces failing results, contact Promega Technical Services to schedule a service visit prior to further use of the instrument.

Note: Wait 1 minute after power cycling the Maxprep® Liquid Handler before starting the software to allow for communication to be re-established.

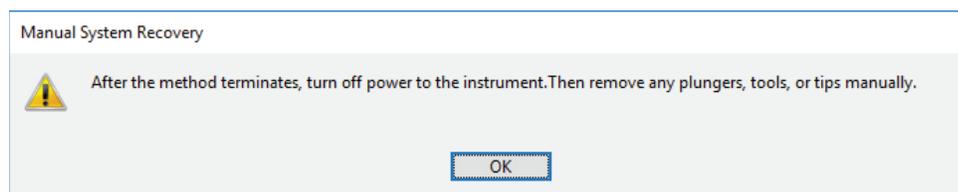


Figure 26. Aborted Method. A message will be displayed after aborting a run indicating that the instrument should be powered off. After turning the instrument off, open the door and manually resolve the crash state as instructed in the *Maxprep® Liquid Handler Technical Manual #TM509*. After any instrument crash, contact Promega Technical Services to determine whether the instrument may require a service visit.

3.8 Instrument Connection Errors

After turning the instrument on, wait at least one minute after the green light on the automated bar code scanner turns on before starting a method run. If the instrument has not fully performed its internal initialization before starting a run, one or more warning messages may be displayed (Figure 27). These warning messages will indicate an error in instrument connection. A message indicating “Unable to ping instrument” is displayed if the instrument is not powered on. Power the instrument on and wait at least one minute after the green light on the bar code scanner turns on before attempting to restart the method. Press the **OK** button, wait several seconds and attempt to start the method again.

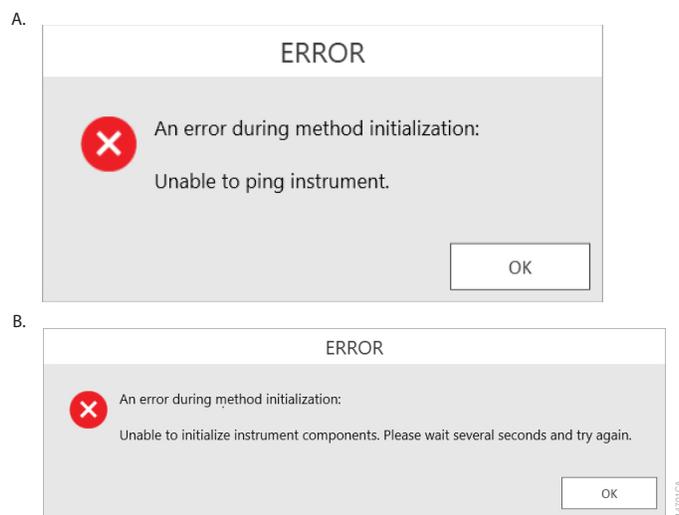


Figure 27. Instrument Connection Errors. Connection errors will be displayed during a run if the instrument has not completed its full internal initialization before a method run is started. The errors shown in **Panels A** and **B** may be displayed. Confirm that the instrument is powered on and has fully initialized, then press the **OK** button, wait several seconds and attempt to restart the method.

4 Variant Method Variables

Administrators should create laboratory-specific variants of QuantiFluor® Dye Quantitation methods for each quantitation type they wish to process. The general process of creating variant methods is discussed in the *Maxprep® Liquid Handler Technical Manual #TM509*. Below is a list of the types of variables that can be adjusted by administrators for quantitation methods. The only variable that can be adjusted by users at run time is 100-fold Dilution of samples. For all other variables listed, the administrator should define the default value for the variable within the displayed minimum and maximum allowable values.

4.1 Controls

These variables define the layout of controls on the reaction plate. Up to three different control types can be used within a run, and multiple well locations can be defined for each control type.



Note: Multiple controls or standards cannot be placed in the same well. Variants with controls that overlap each other or standards will abort at launch.

Setting	Details
Control 1 Concentration	Concentration value of the first control to be used. This value is for informational and reporting purposes only.
Control 1 Name	Name of the first control. Used to identify the control during instrument setup.
Control 1 Well Numbers ¹	Well locations where control #1 should be placed. For multiple control wells, specify the well locations as a comma-delimited list (e.g., 1, 17).
Control 2 Concentration	Concentration value of the second control to be used. This value is for informational and reporting purposes only.
Control 2 Name	Name of the second control. Used to identify the control during instrument setup.
Control 2 Well Numbers ¹	Well locations where control #2 should be placed. For multiple control wells, specify the well locations as a comma-delimited list (e.g., 2, 18).
Control 3 Concentration	Concentration value of the third control to be used. This value is for informational and reporting purposes only.
Control 3 Name	Name of the third control. Used to identify the control during instrument setup.
Control #3 Well Numbers ¹	Well locations where control #3 should be placed. For multiple control wells, specify the well locations as a comma-delimited list (e.g., 3, 19).

¹Well numbering proceeds down columns and across the plate. See Section 7, Appendix, for a visual representation of well numbering in the plate.

4.2 Samples

These variables define how a method should process samples for the quantitation reaction.

Dilute Samples 100-Fold

When checked, the default behavior of the method will be to dilute samples and controls 100-fold prior to quantitation. This is the only variable that can be modified by users at run time. Check the box in the User Modifiable column to allow users to choose whether samples should be diluted or not when running this method. Uncheck the box to prevent users from changing the default behavior specified by the administrator.



Note: Samples and Controls will be diluted 100-fold prior to quantitation setup.

Sample Assay Volume

Indicate the volume of sample to add to each quantitation reaction. Specify the volume in microliters per reaction. The volume of sample must fall within the Minimum and Maximum allowable values specified. The controls and standards will also be transferred to the quantitation reaction at the specified sample volume.

Sample Replicate Number

Specify the number of replicates that should be prepared for each sample. The entire set of samples is placed on the reaction plate as a group, and then the entire group is replicated (Figure 28). As the number of sample replicates increases, the total number of samples that can be processed on a given reaction plate decreases.

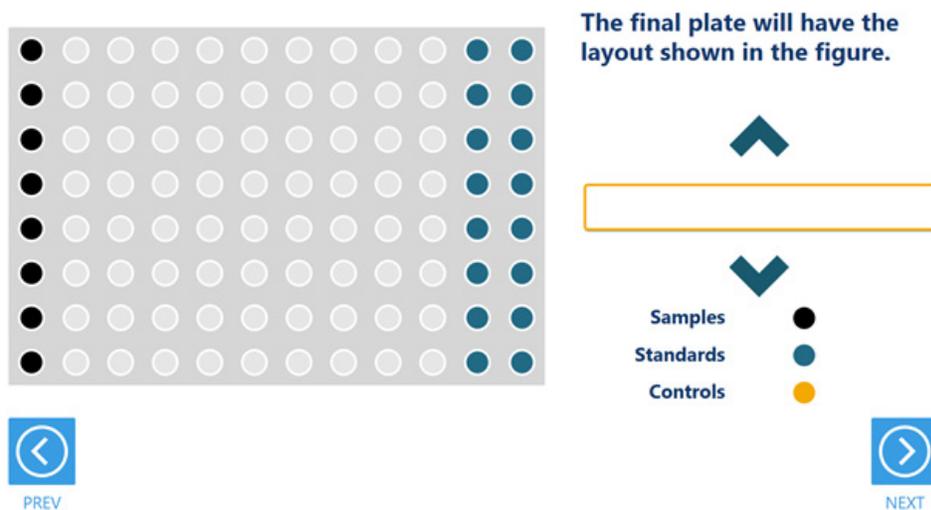


Figure 28. Replicate placement. The entire group of samples is replicated on the reaction plate when replicates are specified in the variant method. Shown here is a group of four samples with a Sample Replicate Number value of two.

4.3 Standards

The standards variables define the concentration of the standard stock, the concentration of each standard, the number of standard curve points and the number of standard curve replicates to use for the quantitation setup.

Prepare Standard Curve

When checked, the system will prepare a standard curve according to the concentration of standards defined.

Concentration of Standards

Up to eight standards can be used to define the standard curve for the quantitation plate. The concentration for each standard to be used can be specified. Units are not provided for standard concentrations to allow for laboratory-specific unit definitions to be used (e.g., ng/ μ l, pg/ μ l, copies/ μ l, etc.); however, all standard concentrations must be specified within the same unit definition. The first standard must have the highest concentration with each subsequent standard decreasing in concentration. The final standard to be used in the standard curve should be a blank and should have a concentration of 0. Any standard positions that are not to be used should be given a concentration of -1. For example, to generate a standard curve with five points and a blank, the highest point being 50ng/ μ l and a 5-fold dilution between points, enter the values below:



Note: The dilution between standard curve points and standard stock should be at least twofold and no greater than 25-fold.

Concentration of 1st Standard	50
Concentration of 2nd Standard	10
Concentration of 3rd Standard	2
Concentration of 4th Standard	0.4
Concentration of 5th Standard	0.08
Concentration of 6th Standard	0
Concentration of 7th Standard	-1
Concentration of 8th Standard	-1

Standard curves will be prepared on the right side of the reaction plate with the final blank curve point in well H12 (96) and the other curve points arrayed sequentially above.

Concentration of Standard Stock

This value specifies the concentration of the standard stock that will be placed on the system. The concentration of the standard stock should be entered in the same unit definition used for the standard curve points. The standard stock must have a concentration equal to or higher than the concentration of the first standard in the standard curve. If the standard stock concentration is the same as the concentration of the first standard curve point, the volume of standard stock used for standard curve generation will be minimized.

Standard Curve Replicates

This value specifies the number of standard curve replicates that should be prepared on the reaction plate. Standard curves are placed on the right side of the reaction plate with each successive standard curve replicate shifted one column to the left. As the number of standard curve replicates increases, the total number of samples that can be processed on a given reaction plate decreases.

5 Troubleshooting

For questions not addressed here, please contact your local Promega Branch, Office or Distributor. Contact information available at: www.promega.com. E-mail: techserv@promega.com

Symptoms	Causes and Comments
Cannot modify sample dilution	<p>The most likely cause of this is that the administrator has disabled the ability of a user to modify this option in the variant method being run.</p> <p>If the administrator has specified that users cannot change the value of a specific variable during a run, it will not be possible to alter the value at run time.</p> <p>Check the settings specified for the variant method that is being run.</p>
The desired labware type is not an available input or output labware option	<p>Many common labware types are available; however, if a specific labware used in the laboratory is not present, contact Promega Technical Services to help resolve this issue.</p>
Cannot advance to the next instrument setup screen	<p>Confirm that all check boxes present on the screen are checked. If at a sample entry screen, confirm that the number of sample positions indicated in the input sample labware exactly matches the number of samples that was set for the run. It is not possible to proceed from a labware setup screen if there is a mismatch between the number of samples that can be processed and the sample number specified.</p>
Instrument error/crash during method run	<p>Double-check that the labware specified during instrument setup exactly matches the labware being used for the method run. Plate movement and pipetting are dependent on precise labware definitions. Do not substitute "similar" labware for the labware specified in the method.</p>
Inaccurate pipetting	<p>Make sure that Daily and Weekly Maintenance methods are being run on a regular basis. They will check that the pipetting functions of the Maxprep® Liquid Handler are working within expected parameters.</p>

6

Related Products

PRODUCT	SIZE	CAT. #
Nunc 2.0ml Deep Well Plates (Sterile)	60/pack	AS9307
2.0ml Deep Well Plates (Non-Sterile)	60/pack	AS9309
Maxwell® RSC Plunger Pack	1 each	AS1670
Maxprep® 1000µl Conductive Disposable Tips, Filtered	40/pack (3,840 tips)	AS9303
Maxprep® 300µl Conductive Disposable Tips, Filtered	60/pack (5,760 tips)	AS9302
Maxprep® 50µl Conductive Disposable Tips, Filtered	60/pack (5,760 tips)	AS9301
Maxprep® 3-Position Reagent Tube Holder	1 each	AS9409
Maxprep® Plunger Holder	1 each	AS9408
Maxprep® Reagent Reservoir, 60ml	28/pack	AS9304
Maxprep® Waste Bags, Clear	100/pack	AS9305
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell® RSC/CSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC/CSC 48 Back Deck Tray	1 each	AS8402
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® CSC Instrument	1 each	AS6000
Maxwell® CSC 48 Instrument	1 each	AS8000
1.1ml, Square-Well V-Bottom Deep Well Plate	25/pack	V6821

7 Appendix

Plate Well Numbering

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

15724MA

Figure 29. Plate map well numbering conversion. On the side and top of the plate image are the alphanumeric row and column labels. Within the wells are the corresponding numeric designations for each well.

8

Summary of Changes

The following changes were made to the 4/24 revision of this document:

1. Added Cat.# AS9105 and AS9205.
2. Updated Section 6.
3. Made minor text edits and formatting changes.

It is the manufacturer's responsibility to provide equipment electromagnetic compatibility information to the customer or user.

It is the user's responsibility to ensure that a compatible electromagnetic environment for the equipment can be maintained in order that the device will perform as intended.

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