Automated Plate Replication

Protocol Guide

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Original Instructions

Agilent Technologies

Notices

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User Guide Part Number

G5591-90001

Edition

Revision A, October 2017

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Automated Plate Replication Protocol Guide

This guide contains the following topics:

- "About this guide" on page 2
- "Protocol description" on page 4
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About this guide

Assumptions

This guide assumes the following:

- The Agilent G5591A Bravo BenchCel Workstation has been installed and is operating correctly.
- The device teachpoints have been set and verified.
- You are familiar with the VWorks Automation Control software.

Safety information

Ensure that you are properly trained in:

- General laboratory safety
- The correct and safe operation of the BenchCel Microplate Handler and Bravo Platform
- Emergency stops

For general safety precautions, intended product use statement, and the list of safety labels, see the *Automation Solutions Products General Safety Guide*.

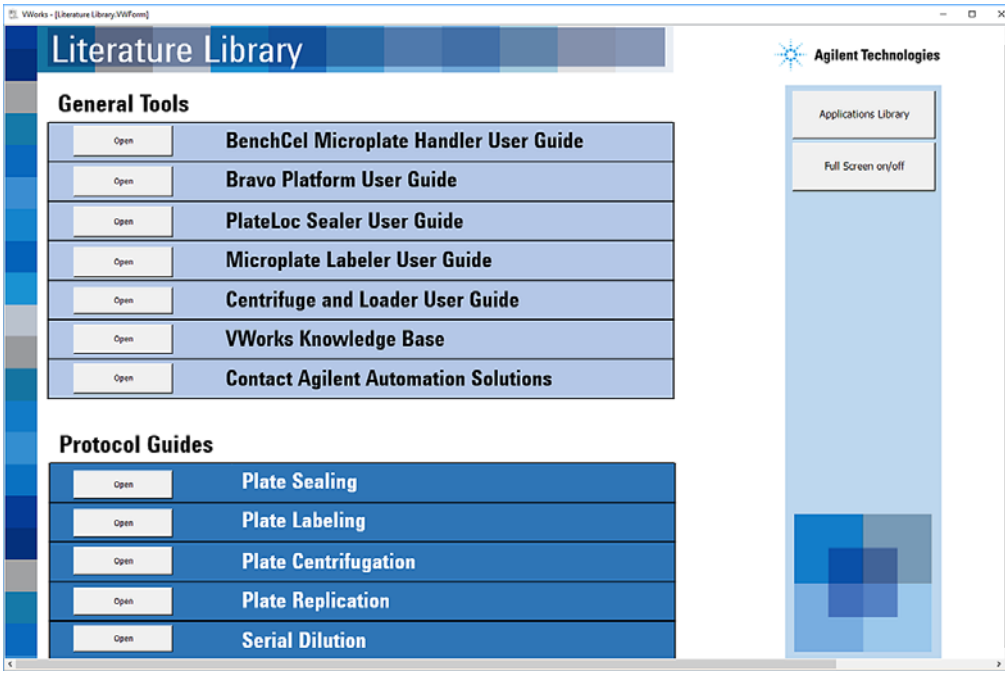
For detailed safety information, see the *BenchCel Microplate Handler User Guide* and the *G5562A, G5563A Bravo Platform User Guide.*

Related user guides

You should use this guide in conjunction with the following guides:

- *Automation Solutions Products General Safety Guide*. Provides general safety information and describes potential safety hazards that you might encounter when using Agilent Automation Solutions products. A copy of this safety guide is included with your shipment.
- *BenchCel Microplate Handler User Guide*. Contains the safety guidelines and describes the installation, setup, operation, and maintenance procedures for the BenchCel Microplate Handler.
- *G5562A, G5563A Bravo Platform User Guide*. Describes the setup, operation, and maintenance procedures for the Bravo Platform.
- *VWorks Automation Control User Guide*. Describes how to create, run, import, and export protocols.

You can access these guides in the **Literature Library** page of the BenchCel Workstations software interface.



You can find the user guides for all the Automation Solutions products in the VWorks knowledge base. You can open the knowledge base in the following ways:

- In the BenchCel Workstations software, go to the **Literature Library** page, and then click **Open** for the **VWorks knowledge base**.
- Within the VWorks software, select **Help > Knowledge Base** or press F1.
- From the Microsoft Windows 10 **All Apps** menu, select **Agilent Technologies > VWorks Knowledge Base**.

You can also find these guides in the online VWorks knowledge base at www.agilent.com/chem/askb.

Protocol description

Plate Replication protocol. The Plate Replication protocol automatically creates a specified number of replicate microplates from a master microplate.

Note: The number (*n*) of microplates that a protocol can process depends on the number of labware racks on the BenchCel Microplate Handler. Depending on the model, the BenchCel Microplate Handler can have two, four, or six labware racks (BenchCel 2R, 4R, or 6R).

Before you start

Hardware requirements

The following figure shows the basic Agilent G5591A Bravo BenchCel Workstation that you use to run this Plate Replication protocol. The BenchCel Microplate Handler in the workstation can be a BenchCel 2R, 4R, or 6R model. The following table describes the primary components.

IMPORTANT If your workstation includes additional devices and you want to use this protocol, the VWorks device file that is linked to the protocol must be edited to include all the workstation devices before you can run the protocol successfully. For instructions on how to modify a device file and link to the protocol, see the *VWorks Automation Control User Guide*.

Figure G5591A Bravo BenchCel Workstation (BenchCel 6R) components

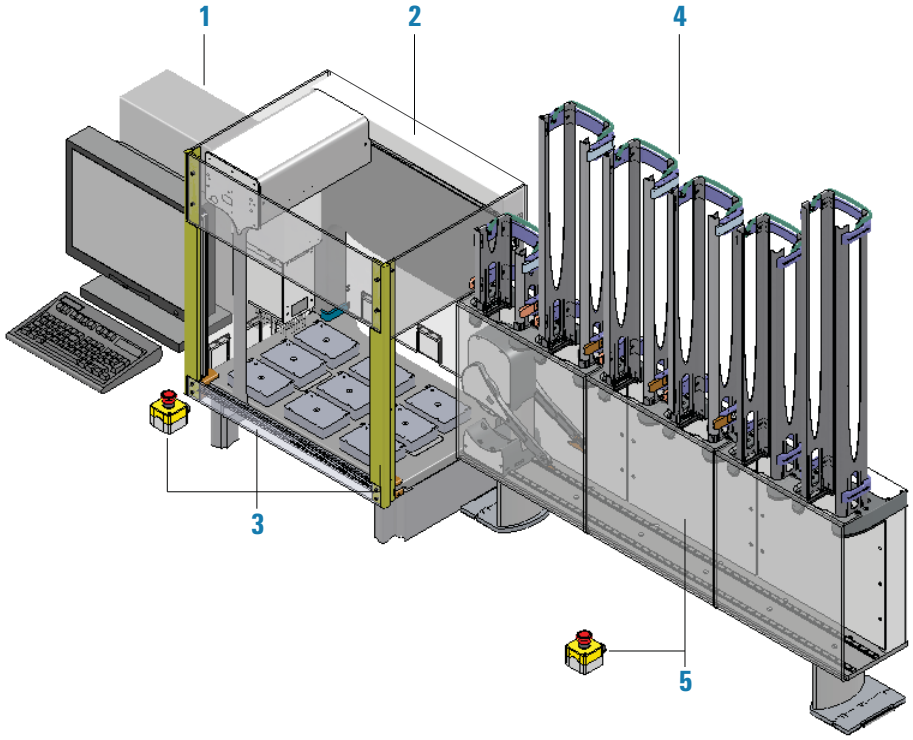


Table Components of the G5591A Bravo BenchCel Workstation

Item	Name	Description	See...
1	Computer and monitor	The controlling computer that runs the VWorks Automation Control software.	*VWorks Automation Control User Guide*
2	Bravo Platform	The automated liquid handler with nine deck locations for labware. The Bravo includes a gripper assembly that moves labware from one deck location to another. **IMPORTANT** This protocol requires the 96LT Head to be installed on the Bravo Platform.	*G5562A, G5563A Bravo Platform User Guide*
3	Bravo safety equipment	The equipment that protects users from potential moving-parts hazards, including emergency-stop pendant, Light Curtain, and shields.	*G5562A, G5563A Bravo Platform Safety and Installation Guide*
4	BenchCel Microplate Handler	The device that stores stacks of labware and moves labware to and from the Bravo Platform. Depending on the BenchCel model, two, four, or six labware racks are installed on the stackers. • BenchCel 2R has two stackers for labware racks. • BenchCel 4R has four stackers for labware racks. • BenchCel 6R has six stackers for labware racks. The labware rack height can be 250-, 660-, or 860-mm.	*BenchCel Microplate Handler User Guide*
5	BenchCel safety equipment	An emergency-stop pendant and a shield in front of the BenchCel robot protect operators from moving-parts hazards.	*BenchCel Microplate Handler User Guide*

Software requirements

The minimum software requirements for running the protocol are as follows:

- Microsoft Windows 10 64-bit operating system
- VWorks Automation Control software 13.1
- BenchCel Workstations software 1.0

The BenchCel Workstations software includes the device profiles for connecting to your devices and the files required to run the BenchCel Workstation protocols.

Labware requirements

CAUTION Use only the labware specified, and place them at the locations specified in the instructions. Using different labware or placing labware at an unapproved location can cause a collision resulting in equipment damage.

- **Full tip boxes of Agilent 250-µL pipette tips**. The number of tips boxes to be used should be equivalent to the number of source microplates being processed.

  Note: A 660-mm labware rack can hold up to 11 tip boxes.
- **Source and destination microplates**. The following table lists the labware options that you can choose from for this protocol.

Table Labware options for source and destination microplates

Labware entry in VWorks list	Labware name	Manufacturer part number
96 Costar 3961 PP 2ml assay block	96-Well Clear V-Bottom 2mL Polypropylene Deep Well Plate	Corning Costar 3961
96 Greiner 655101 PS Clr Rnd Well Flat Btm	96-Well Microplate, Polystyrene, Round Well, Flat Bottom	Greiner 655101
96 EK 2460 PP Rnd Well U Btm	96 Target Well Plates, 500 µl, U-Bottom, Clea	E&K Scientific EK-2460
96 Nunc Deep Well 1 mL	Nunc 96 DeepWell plate, sterile, 1.3-mL volume	Thermo Scientific 260251
96 Seahorse Storage Plate 2ml Square Pyramid	Seahorse 96-well PD-702 polypropylene storage microplate, 2ml/square well, pyramid bottom	Agilent Technologies 201379100
384 Greiner 781101 PS clr flt btm	Greiner 384 well plate, polystyrene, clear flat bottom	Greiner 781101
1536 Greiner 782076 blk sqr well flt btm	Greiner FLUOTRAC 200 1536-well plate, black polystyrene, flat bottom	Greiner 782076

Note: The labware that you use must have a corresponding labware definition in the VWorks software. In the labware definition, the BenchCel properties should specify the Stack holding method: Hold with stacker grippers. For detailed instructions on how to create or edit labware definitions, see the *VWorks Automation Control Setup Guide*.

Starting and setting up the workstation

Starting up the workstation

To start the workstation:

1 At the Robot Disable Hub ensure that the LIGHT CURTAIN switch remains set to ENABLE.

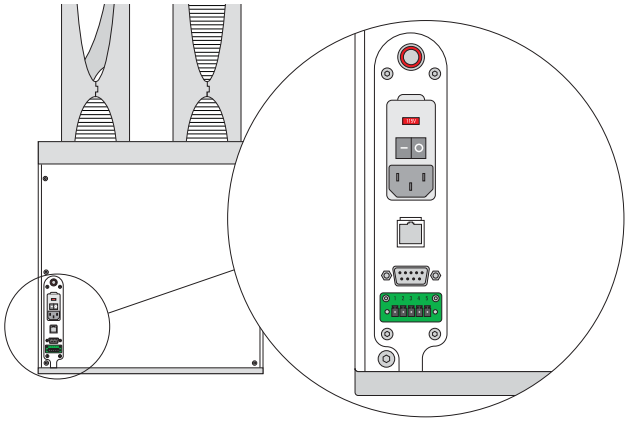
2 Start up the BenchCel Microplate Handler as follows:

a Position the BenchCel robot head underneath a stacker, and place the gripper arms to the left facing the Bravo Platform.

b Turn on the BenchCel air supply.

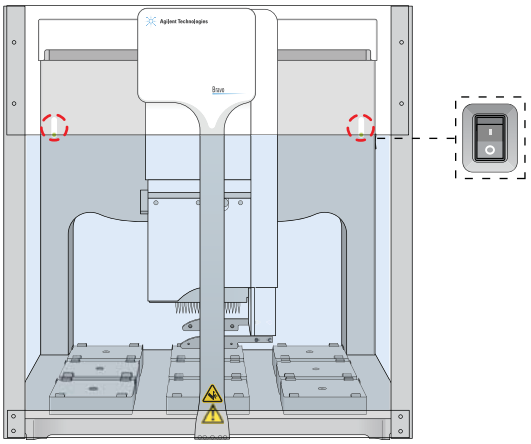
c Turn on the BenchCel power.

Figure BenchCel rear panel



d Wait for the BenchCel head to finish the homing routine.

3 Turn on the Bravo Platform. The front panel status lights turn blue.

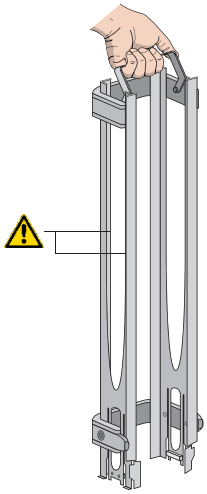


4 Turn on the computer and monitor. Wait for the Microsoft Windows operating system to finish starting up.

5 At the BenchCel Microplate Handler, install the labware racks on the stackers. See the *BenchCel Microplate Handler User Guide*.

IMPORTANT Make sure the BenchCel power and compressed air are turned on before you install or uninstall a labware rack.

Figure Labware rack, front-load



Opening the protocol

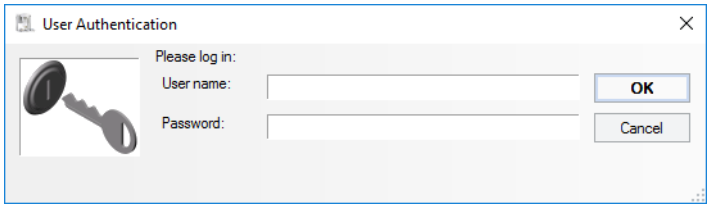
IMPORTANT Ensure that the VWorks software is closed before you start the BenchCel Workstations software.

To open the protocol:

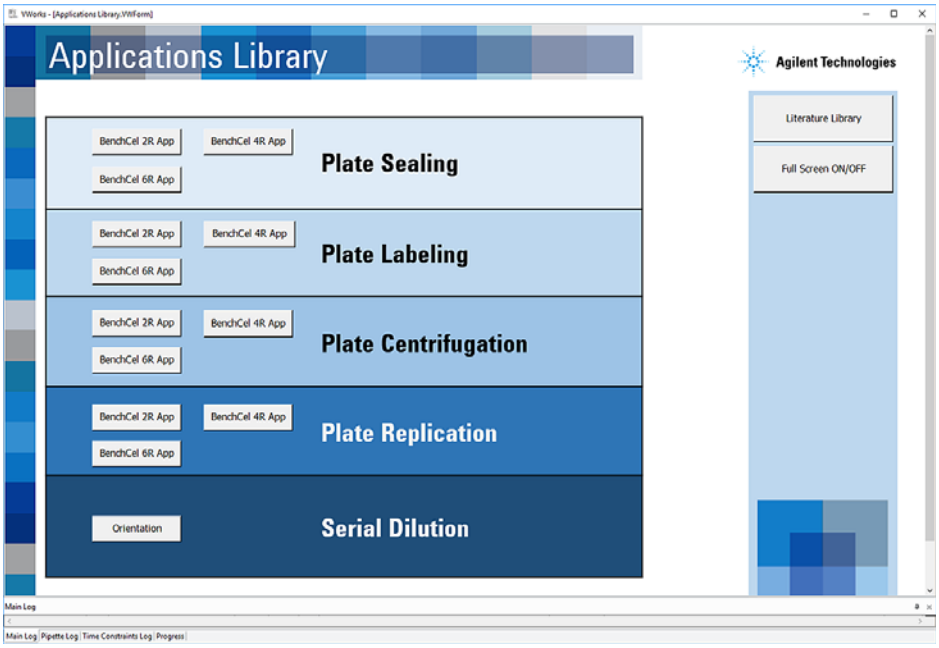
1 Start the BenchCel Workstations software .

2 Click **App Library**. The VWorks software starts.

3 When the **User Authentication** dialog box opens, type your VWorks user name and password, and then click **OK**.



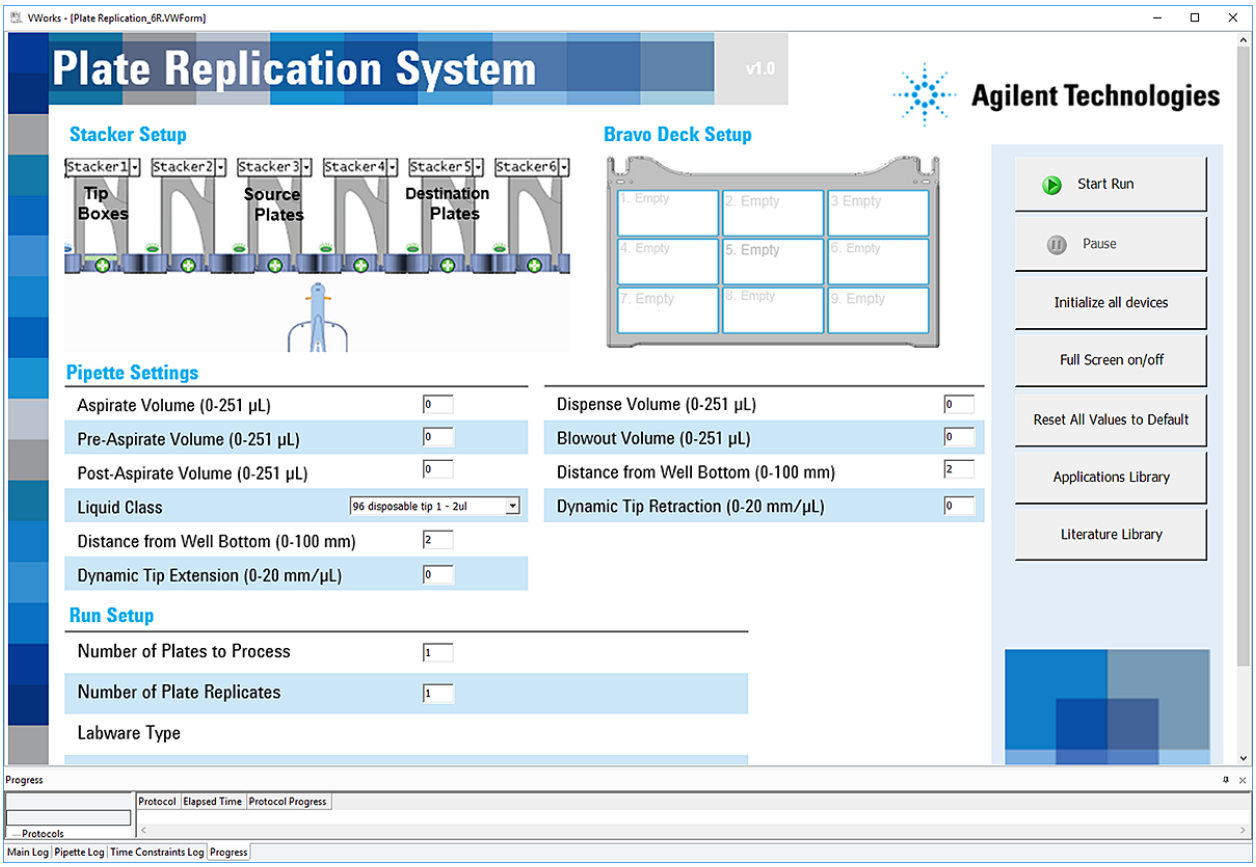
The **Applications Library** form opens.



4 In the **Plate Replication** area, click the button that corresponds to your configuration: BenchCel 2R, BenchCel 4R, or BenchCel 6R.

The Plate Replication form opens in the VWorks window.

Figure VWorks window displaying the Plate Replication form for BenchCel 6R



Setting up the protocol

Specifying the Pipette Settings

Ensure that the total Dispense volume, including air gaps and liquid, is equivalent to the total Aspirate volume, including air gaps and liquid.

To specify the pipette settings:

1 *Optional.* In the form, click **Reset All Values to Default** to set all the form settings to their defaults.

2 *Optional.* If you want to view the VWorks toolbars and menus, click **Full Screen on/off** to change the scale of the form display within the VWorks window.

3 Under **Pipette Settings**, set the values for the following Aspirate parameters:

Parameter	Description
Aspirate Volume (0–251 µL)	The volume of liquid to be drawn into each pipette tip.
Pre-Aspirate Volume (0–251 µL)	The volume of air to be drawn before the pipette tips enter the liquid.
Post-Aspirate Volume (0–251 µL)	The volume of air to be drawn after the liquid is drawn.
Liquid Class	The pipetting speed and accuracy. Choose from the following options depending on your volume requirements: • 96 disposable 1–2 µL • 96 disposable 2–50 µL • 96 disposable 51–200 µL **IMPORTANT** To ensure consistent pipetting, always select a liquid class for liquid-handling tasks.
Distance from Well Bottom (0–100 mm)	The distance between the end of the pipette tips and the well bottoms at the end of the Aspirate task.

Parameter	Description
Dynamic Tip Extension (0–20 mm/µL)	The rate at which the pipette head moves during the Aspirate task. The software calculates the distance over which the tips will move. To move the tips: • *At the same rate as the volume change.* Calculate dynamic tip extension (DTE) as follows: DTE = (well depth)/(well vol) = 1/A, where A is the cross-sectional area of a well with straight walls • *Faster than the volume change.* DTE > 1/A • *Slower than the volume change.* DTE < 1/A The starting and ending positions can be calculated as follows: $(V_{aspirated} * DTE) + Distance_{well\ bottom}$ *Note:* Instead of a negative aspirated volume, the software automatically moves downward toward the well bottom with each aspirate action.

4 Set the values for the following Dispense parameters:

Parameter	Description
Dispense Volume (0–251 µL)	The volume of liquid to be dispensed from each pipette tip.
Blowout Volume (0–251 µL)	The volume of air to dispense after the main volume has been dispensed while the tips are still in the wells. Typically, the blowout volume is the same as the pre-aspirate volume.
Distance from Well Bottom (0–100 mm)	The distance between the end of the pipette tips and the well bottoms at the start of the Dispense task.
Dynamic Tip Extension (0–20 mm/µL)	The rate at which to raise the pipette head during the Dispense task. To move the tips: • *At the same rate as the volume change.* Calculate dynamic tip retraction (DTR) as follows: DTR = (well depth)/(well vol) = 1/A, where A is the cross-sectional area of a well with straight walls • *Faster than the volume change.* DTR > 1/A • *Slower than the volume change.* DTR < 1/A The starting and ending positions can be calculated as follows: $(V_{dispensed} * DTR) + Distance_{well\ bottom}$

Specifying the run setup

To specify the run setup:

1 In the **Run Setup** area:

   a In the **Number of Plates to Process** box, type the value for the total number of Source plates.

   b In the **Number of Plate Replicates** box, type the value for the number of replicates per Source plate.

2 Under **Labware Type**, select the labware for the following:

   a **Source.** The labware you are using for the master microplates.

   b **Destination.** The labware you are using for the replicate microplates.

Setting up the labware for the run

For instructions on how to handle the labware racks and fill them with labware, see the *BenchCel Microplate Handler User Guide*.

CAUTION Improperly seated labware can cause a hardware collision, resulting in equipment damage. Ensure that all labware are properly seated and in the correct orientation within the BenchCel labware racks and the alignment features of the Bravo platepads.

IMPORTANT Fill the source microplates immediately before run time to minimize evaporation.

About stacking the labware on the Bravo deck

IMPORTANT The labware stack height maximum is 100 mm on the Bravo deck for a standard platepad.

For the BenchCel 2R and 4R protocols, you stack labware on the Bravo deck. The number of microplates that a stack can contain varies depending on the type of microplate. To determine the height limit for your labware stack, refer to the Stacking Thickness value for the given labware definition. For details on labware definitions, see the *VWorks Automation Control Setup Guide*.

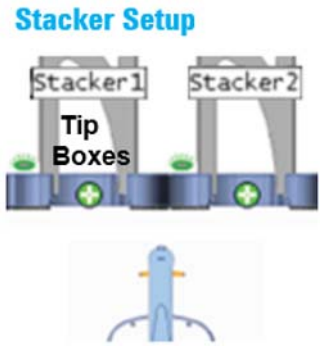
For, example, if you have 2 Source plates and want to create 3 replicates each, you would require 6 Destination plates. A stack of 6 Greiner 655101 microplates (14.62-mm stacking thickness) is within the stacking height limit. However, a stack of only 2 Costar 3961 microplates (41.3-mm stacking thickness) is within the stacking height limit. The protocol will pause at the appropriate time, if applicable, for you to replenish the microplate stack.

CAUTION To prevent a potential hardware collision, ensure that the labware stack is fully seated on the Bravo platepad.

BenchCel 2R workstation labware setup

To set up the labware for the BenchCel 2R workstation:

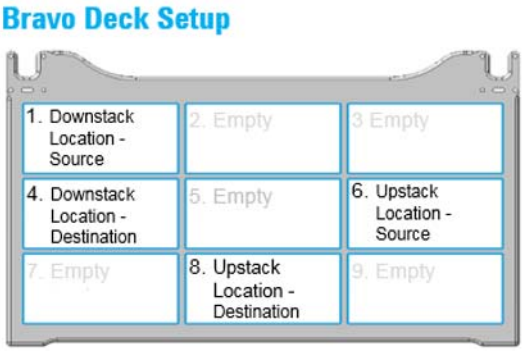
1 At BenchCel stacker 1, fill the labware rack with the required number of full tip boxes for your run.



Verify BenchCel stacker 2 is available for storing the used tip boxes.

Note: During the protocol, the BenchCel robot will downstack the tip box to Bravo deck location 3 for Tips On and Tips Off operations. The BenchCel robot will upstack the used tip box to stacker 2.

2 In the form, locate the **Bravo Deck Setup** area, and then place the specified labware at the designated locations:



- At deck location 1 (Downstack Location), place the Source (master) plates.

  Note: During the protocol, the Bravo gripper will downstack the top Source plate and move it to deck location 2 for processing. The Bravo gripper will stack the used Source plates at deck location 6.

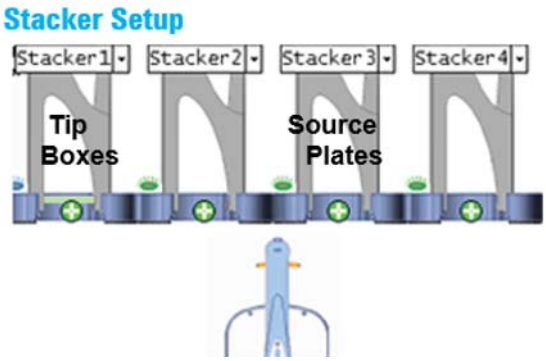
- At deck location 2 (Downstack Location), place the Destination (replicate) plates.

  Note: During the protocol, the Bravo gripper will downstack the top Destination plate and move it to deck location 5 for processing. The Bravo gripper will stack the completed Destination plates at deck location 8.

BenchCel 4R workstation labware setup

To set up the labware for the BenchCel 4R workstation:

1 In the form, locate the **Stacker Setup** area, and then fill the labware racks in the designated BenchCel stackers with the appropriate labware:



a **Stacker 1**. Fill the labware rack with the enough full tip boxes to meet the requirements of your run.

Note: During the protocol, the BenchCel device will downstack the tip box to Bravo deck location 3. The Bravo gripper will move the tip box to deck location 2 for Tips On and Tips Off operations. The BenchCel will upstack the used tip boxes to stacker 2.

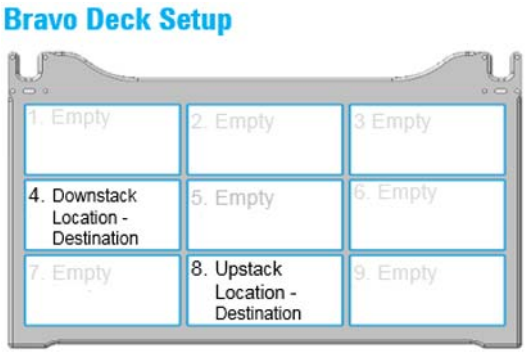
b **Stacker 3.** Fill the labware rack with the total number of prepared Source (master) plates to meet the requirements of your run.

Ensure that these microplates contain the liquid to be transferred to the Destination plates.

Note: During the protocol, the BenchCel device will downstack the Source plate to Bravo deck location 3. The Bravo gripper will move the Source plate to deck location 6 for pipetting operations. The BenchCel will upstack the used Source plates to stacker 4.

c **Stackers 2 and 4**. Verify that the labware racks are available for storing the used tip boxes and Source plates.

2 At the Bravo deck, place the stack of Destination (replicate) plates at deck location 4 (Downstack Location).

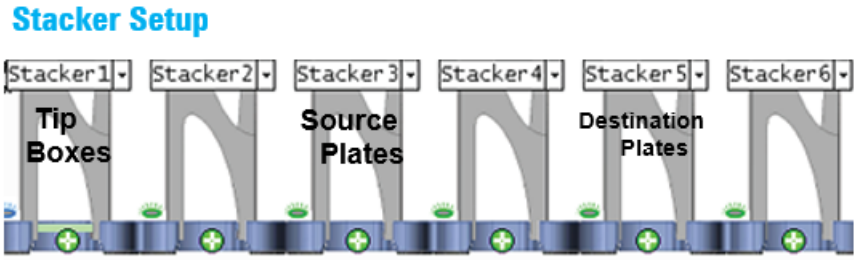


Note: During the protocol, the Bravo gripper moves the Destination plate to deck location 5. After filling the Destination plate, the Bravo gripper moves it to deck location 8.

BenchCel 6R workstation labware setup

To set up the labware for the workstation:

1 In the form, locate the **Stacker Setup** area, and then fill the labware racks in the designated BenchCel stackers with the appropriate labware:



a **Stacker 1**. Fill the labware rack with the enough full tip boxes to meet the requirements of your run.

Note: During the protocol, the BenchCel device will downstack the tip box to Bravo deck location 3. The Bravo gripper will place the tip box at deck location 1 for Tips On and Tips Off operations. The BenchCel will upstack the used tip boxes to stacker 2.

b **Stacker 3.** Fill the labware rack with the total number of prepared Source (master) plates to meet the requirements of your run.

Note: During the protocol, the BenchCel robot will downstack the Source plate to the Bravo deck. The Bravo gripper will place the Source plate at deck location 6 for pipetting operations. The BenchCel will upstack the used Source plates to stacker 4.

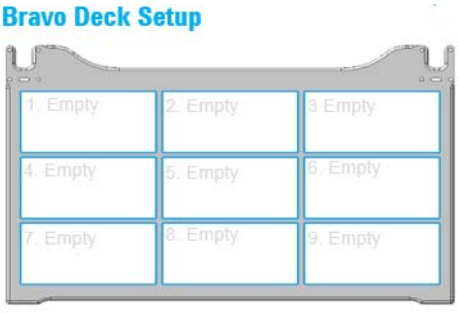
c **Stacker 5.** Fill the labware rack with the total number of Destination (replicate) plates to meet the requirements of your run.

Ensure that you have a sufficient number of Destination plates for the run. For, example, if you have 2 Source plates and want to create 3 replicates each, you would require 6 Destination plates.

Note: During the protocol, the BenchCel robot will downstack the Destination plate to the Bravo deck. The Bravo gripper will place the Destination plate at deck location 2 for pipetting operations. The BenchCel will upstack the filled microplates to stacker 6.

d **Stackers 2, 4, and 6**. Verify that the labware racks are available for storing the used tip boxes, Source plates, and Destination plates.

2 Ensure that the Bravo deck is clear of any labware.



Running the protocol

Before you start the run

WARNING **When you initialize the devices, the Bravo head and tie bar can move and the BenchCel robot can move. To prevent potential injury, keep clear of the device while it is in motion.**

CAUTION A collision can occur if an object is placed in the path of the BenchCel gripper or Bravo head. To prevent potential equipment damage, ensure that the path is clear of objects, such as extra labware, that could cause a potential collision.

IMPORTANT Ensure that the Bravo 96LT head is mounted on the Bravo Platform and that no pipette tips are mounted on the head.

About performing a mock run (optional)

If you are unfamiliar with the protocol and would like to see how it operates and troubleshoot problems before running it with valuable samples and reagents, you can perform a mock run. A mock run uses empty or water-filled labware.

You prepare for a mock run the same way you would prepare for a real protocol run, except that you use empty labware for a totally dry run or labware containing water for a wet run.

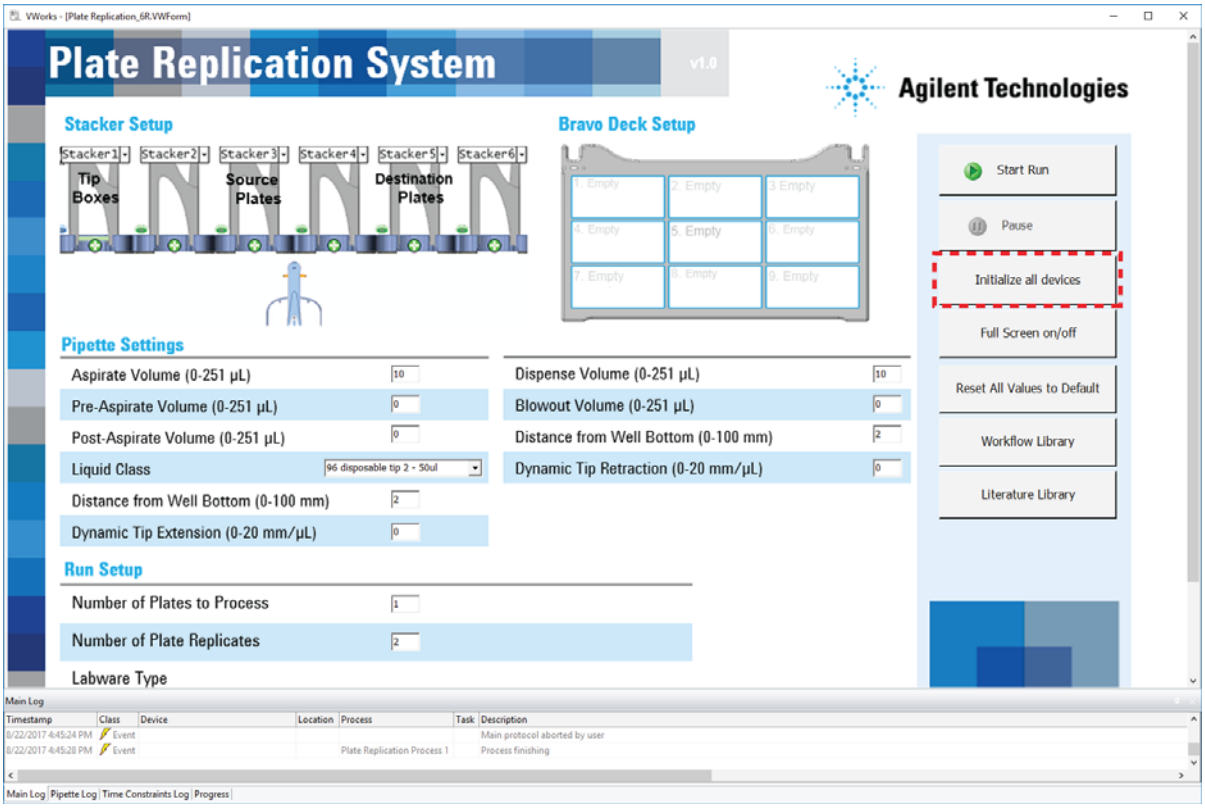
Starting and monitoring the protocol run

To start and monitor the protocol run:

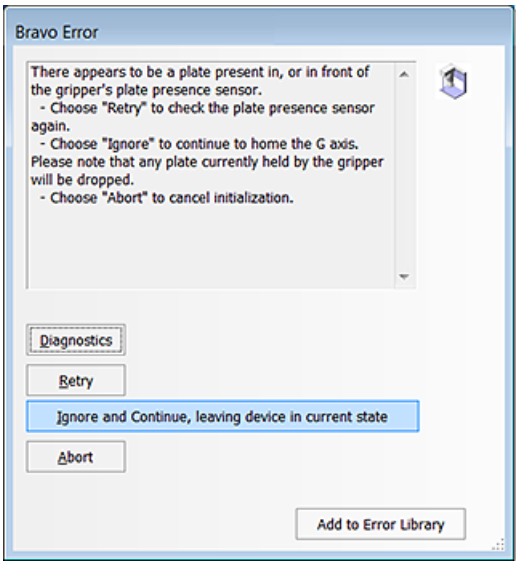
1. Confirm that the physical layout of the hardware and labware matches the selections in the form.
2. If the workstation devices are already initialized, you can skip to step 5.

   If the devices are not yet initialized, click **Initialize all devices** in the Plate Replication form.

   The initialization process establishes communication with the BenchCel Microplate Handler and the Bravo Platform and homes the devices.



3 If the **Bravo Error** message appears stating **There appears to be a plate present**, verify that Bravo gripper is not holding labware, and then click **Ignore and Continue** to continue the initialization process.



4 If the **Verify that it is safe to home the W-axis** message appears, click **Retry** to continue homing the pipetting axis (*w*-axis).

5 In the **Plate Replication** form, click Start Run. The protocol run starts.

Note: A run of 1 Source plate, 1 tip box, and 5 Destination plates (replicates) should take approximately 7 minutes.

To monitor the progress of the run, check the **Progress** tab at the bottom of the VWorks window.

At the end of the run, the BenchCel stacker grippers automatically release the labware so that you can unload the labware.

A protocol complete message appears.

If you close the protocol form, a message asks if you would like to save changes to the form:

- Click **Yes** to save the parameter settings in the form.
- Click **No** to keep the previously saved parameter settings in the form.

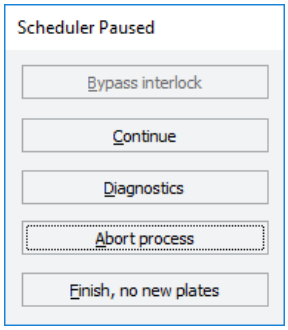
The next time you open the form, it displays the last set of saved parameter settings. If you want to return to the default values, click **Reset All Values to Default**.

Pausing and continuing the run

To pause and continue the run:

1 In the **Plate Replication** form, click Pause.

The task currently in progress finishes before the protocol pauses. The Scheduler Paused dialog box opens.



2 When you are ready to resume the run, click **Continue** in the **Scheduler Paused** dialog box.

For details about the other options in the Scheduler Paused dialog box, see the *VWorks Automation Control User Guide*.

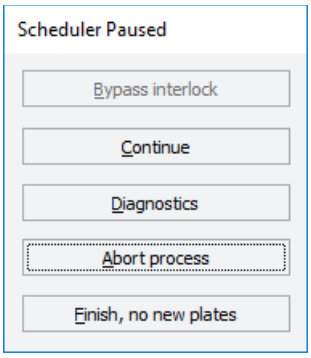
Stopping in an emergency

CAUTION You might not be able to resume a protocol after an emergency stop. Do not use an emergency stop to pause a run. To pause and continue a run, use the Pause button.

To stop in an emergency:

1 Press the red button on one of the emergency-stop pendants.

The Scheduler Paused dialog box opens.



To recover from an emergency stop:

1. Clear any obstacles from the Bravo Light Curtain. To reactivate the pendant, turn the red button clockwise. The spring-loaded button pops up.

2. Remove any dropped labware from the workstation. Remove the labware from the BenchCel gripper and Bravo Platform.
3. In the Scheduler Paused dialog box, select the appropriate command. For details, see the *VWorks Automation Control User Guide*.

   Depending on the state of the workstation when the emergency stop was activated, the run may not be recoverable.

Agilent Technologies

Protocol Guide

G5591-90001 Revision A

October 2017