

Automated Plate Centrifugation

Protocol Guide

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Original Instructions



Agilent Technologies

Notices

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A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.



Automated Centrifugation Protocol Guide

This guide contains the following topics:

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About this guide

This guide describes how to run the Automated Plate Centrifugation protocol using an Agilent G5590A BenchCel Workstation that includes the PlateLoc Sealer over Microplate Centrifuge with Loader and the BenchCel Workstations software. For details on how create a different protocol, see the [VWorks Automation Control User Guide](#).

Assumptions

This guide assumes the following:

- The Agilent G5590A BenchCel Workstation with PlateLoc Sealer over Microplate Centrifuge with Loader has been installed and is operating correctly.
- The BenchCel Workstations software has been installed.
- The device teachpoints have been set and verified.
- You are familiar with the devices in the workstation and the VWorks Automation Control software.

Safety information

Ensure that you are properly trained in:

- General laboratory safety
- The correct and safe operation of the workstation devices
- Emergency stops

For general safety precautions, intended product use statement, and the list of safety labels, see the [Automation Solutions Products General Safety Guide](#).

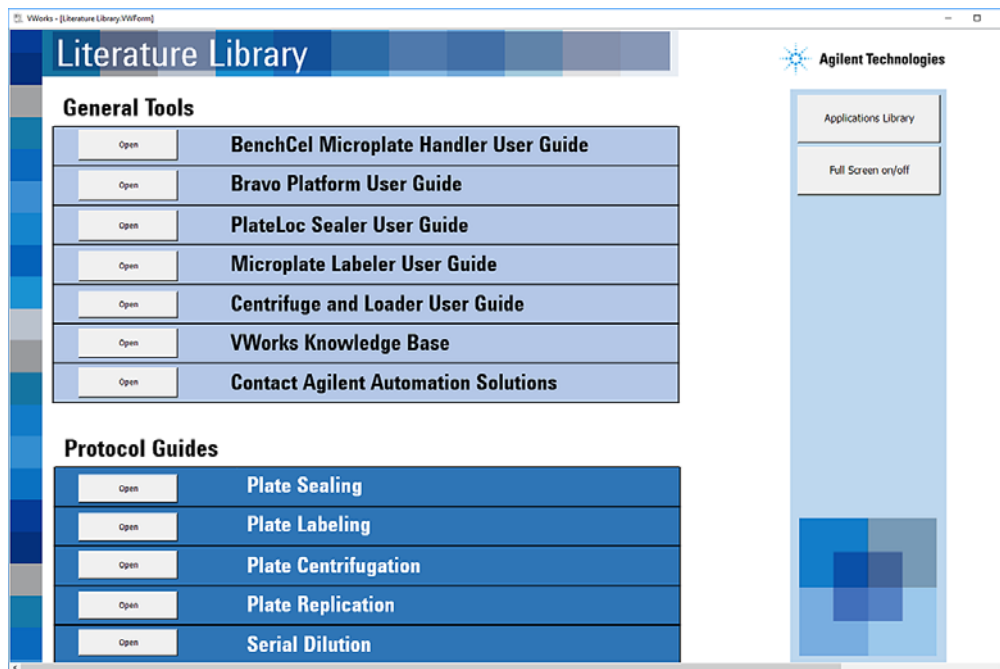
For detailed safety information, see the [BenchCel Microplate Handler User Guide](#) and the [Microplate Centrifuge with Loader User Guide](#).

Related user guides and where to find them

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.
- [BenchCel Microplate Handler User Guide](#). Contains the safety guidelines and describes the installation, setup, operation, and maintenance procedures for the BenchCel Microplate Handler.
- [Microplate Centrifuge with Loader User Guide](#). Contains the safety guidelines and describes the installation, setup, operation, and maintenance procedures for the Microplate Centrifuge with Loader.
- [VWorks Automation Control User Guide](#). Describes how to create, run, import, and export protocols.
- [VWorks Automation Control Setup Guide](#). Explains how to define labware, specify pipetting speed and accuracy, manage labware in storage, and manage user accounts.

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 Centrifugation protocol. The Plate Centrifugation protocol automatically centrifuges up to n microplates in a single automated protocol run.

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 (2R), four (4R), or six (6R) labware racks.

Before you start

Hardware requirements

The following figure shows the G5590A BenchCel Workstation configuration that you use to run the Plate Centrifugation protocol. The following table describes the primary components in the workstation. The BenchCel Microplate Handler in the workstation can be a BenchCel 2R, 4R, or 6R model

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 G5590A BenchCel Workstation (BenchCel6R) with PlateLoc over Centrifuge

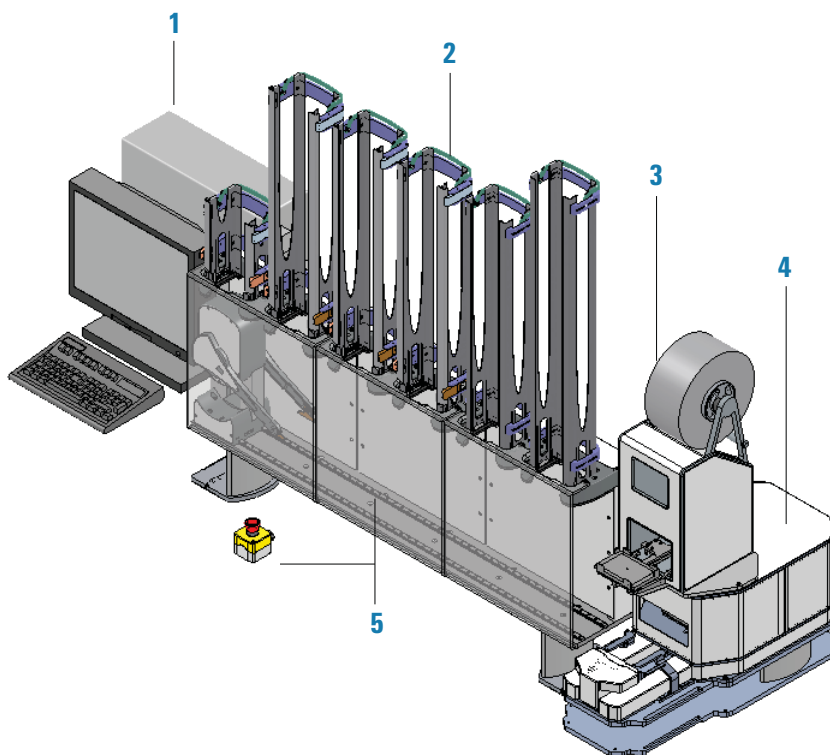


Table Components of the G5590A BenchCel Workstation with PlateLoc over Centrifuge

| Item | Name | Description | See... |
|------|------------------------------------|---|--|
| 1 | Computer and monitor | The controlling computer that runs the VWorks Automation Control software. | <i>VWorks Automation Control User Guide</i> |
| 2 | BenchCel Microplate Handler | <p>The device that stores stacks of labware and moves labware to and from the other devices in the workstation.</p> <p>Depending on the BenchCel model, two, four, or six labware racks are installed on the stackers:</p> <ul style="list-style-type: none"> • BenchCel 2R has two stackers for labware racks. • BenchCel 4R has four stackers for labware racks. • BenchCel 6R has six stackers for labware racks. <p>The rack height can be 250-, 660-, or 860- mm.</p> | <i>BenchCel Microplate Handler User Guide</i> |
| 3 | PlateLoc Thermal Microplate Sealer | <p>The robot-accessible automated microplate sealer (PlateLoc Sealer) that applies a sealing material on top of microplates to seal individual wells.</p> <p>IMPORTANT The PlateLoc Sealer is not used during this Plate Centrifugation protocol. But the VWorks device file that the protocol uses references all the automation devices in the workstation.</p> | <i>PlateLoc Thermal Microplate Sealer User Guide</i> |
| 4 | Microplate Centrifuge with Loader | <p>The automated centrifuge with a robotic microplate handler.</p> <p>After the BenchCel robot places a microplate onto the Loader plate stage, the Loader gripper picks up the microplate and moves it into the Centrifuge. The Centrifuge has two buckets, each capable of carrying a single microplate.</p> | <i>Microplate Centrifuge with Loader User Guide</i> |
| 5 | BenchCel safety equipment | An emergency-stop pendant and a shield in front of the BenchCel robot protect operators from moving-parts hazards. | <i>BenchCel Microplate Handler User Guide</i> |

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 VWorks device file and the profiles for connecting to your devices, and the files required to run the provided BenchCel Workstation protocols.

Labware requirements

The following table lists the labware options that you can choose from when running the Plate Centrifugation protocol.

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

Table Labware options

| 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 ul, 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 |

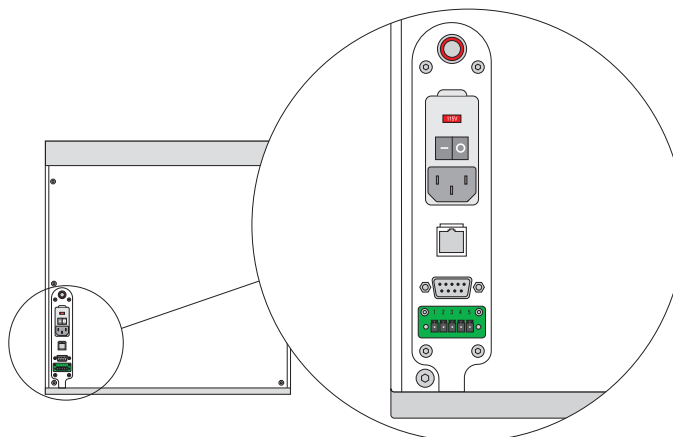
*The labware that you use must have a corresponding labware definition in the VWorks software. In the labware definition, the BenchCel properties should specify the Stack holding method: Hold with stacker grippers. For detailed instructions on how to create or edit labware definitions, see the [VWorks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

Starting up the workstation

To start the workstation:

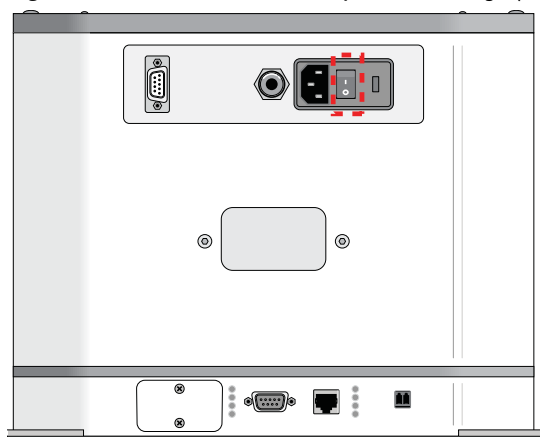
- 1 Start up the BenchCel Microplate Handler as follows:
 - a Position the BenchCel robot head underneath a stacker, and place the gripper arms to the right, facing the Microplate Centrifuge.
 - b Turn on the BenchCel air supply.
 - c Turn on the BenchCel power.

Figure BenchCel rear panel

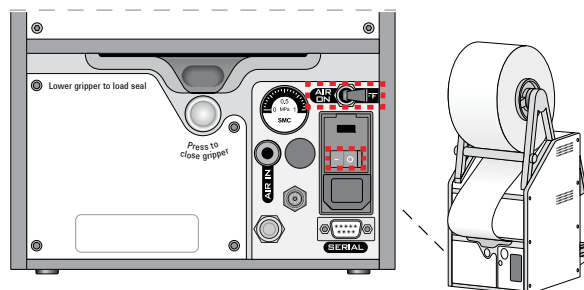


- d Wait for the BenchCel head to finish the homing routine.
- 2 Start up the Microplate Centrifuge with Loader as follows:
 - a Verify that the air supply is turned on.
 - b On the back of the Microplate Centrifuge, press the power switch to the on (I) position.

Figure Power switch on Microplate Centrifuge (rear view)



- 3 Start up the PlateLoc Sealer as follows:
 - a Press the on/off switch to the on position (I).
 - b Push the **AIR ON/OFF** switch to the **ON** position.



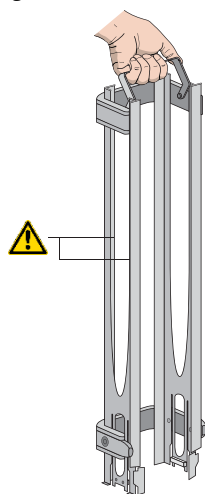
When you turn on the air, the PlateLoc Sealer door opens. The door remains open while the device is in idle mode, until you load a microplate.

IMPORTANT Even though the protocol does not use the PlateLoc Sealer, the VWorks software will display an error message if communication cannot be established with a device in the device file.

- 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

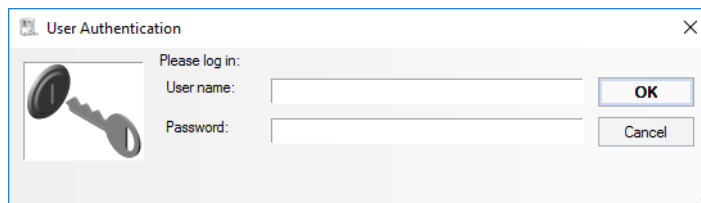
IMPORTANT Ensure that the VWorks software is closed before you start the BenchCel Workstations software.

To open the protocol:

- 1 Start the BenchCel Workstations software .



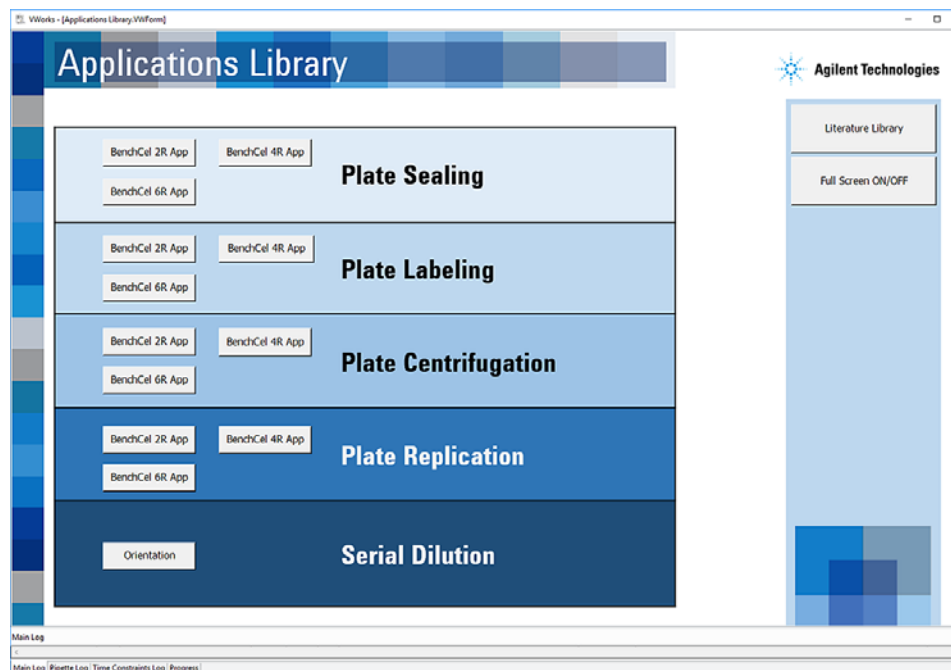
- 2 Click **App Library**. The VWorks software starts.
- 3 When the **User Authentication** dialog box opens, type your VWorks user name and password, and then click **OK**.



The Applications Library form opens.

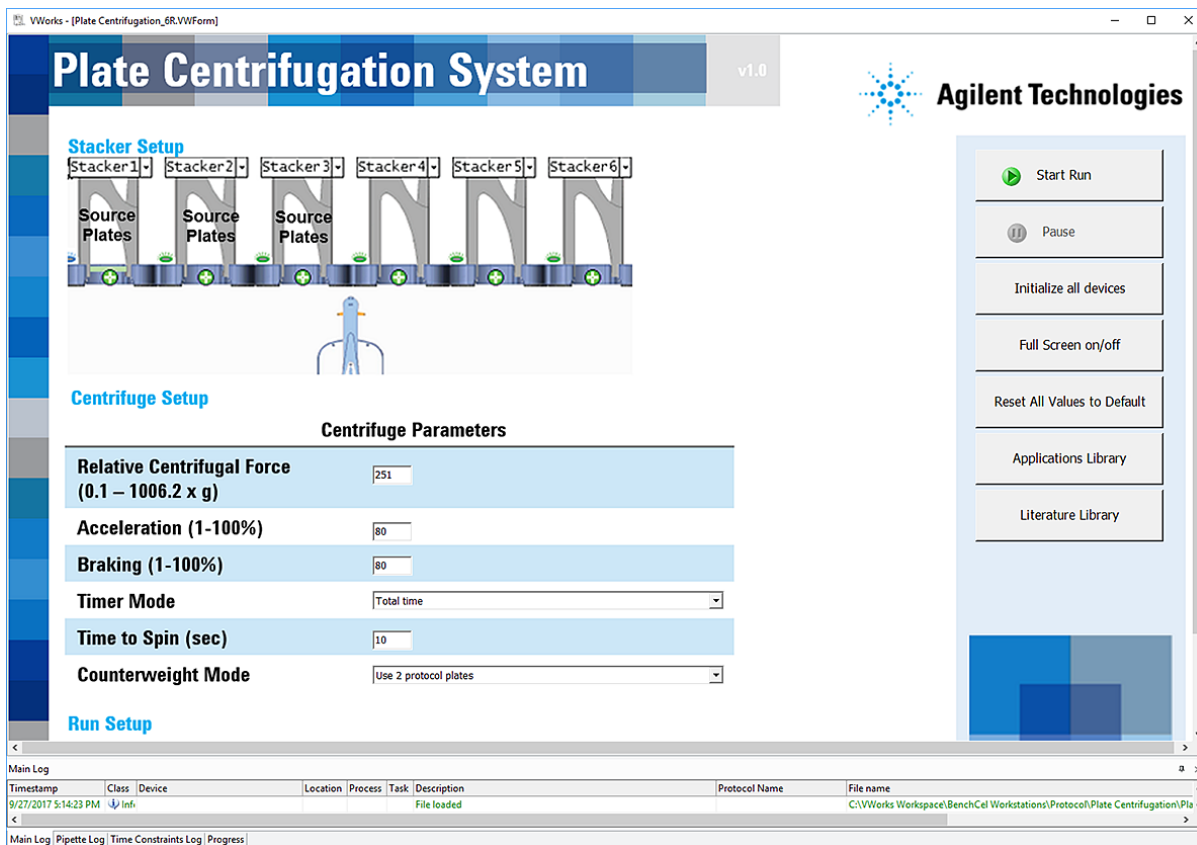
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Opening the protocol



- 4 In the **Plate Centrifugation** area, click the button that corresponds to your configuration: BenchCel 2R App, BenchCel 4R App, or BenchCel 6R App. The Plate Centrifugation form opens in the VWorks window.

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



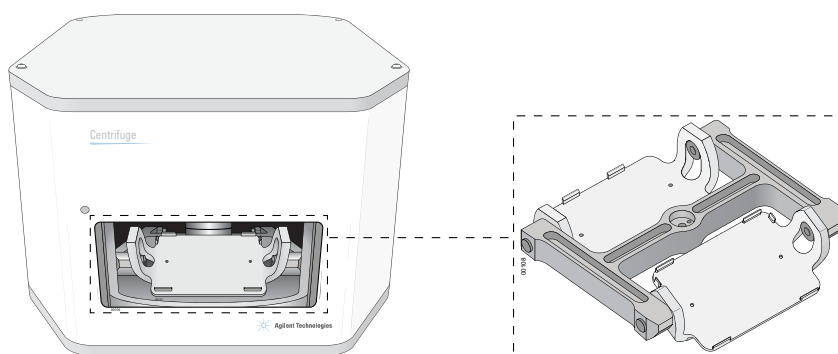
Setting up the protocol

Before you start



WARNING Each Centrifuge bucket can hold a microplate or counterweight that weighs up to 250 g (8.82 oz). Placing heavier microplates or counterweights in the Centrifuge can cause the device to malfunction during operation, damaging the device and causing severe injury.

The contents of the Centrifuge buckets must be within 10 g of each other to remain balanced during the spin. Unbalanced buckets can cause a spin error. Centrifuge buckets



Specifying the centrifugation settings

To specify the centrifugation 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 In the **Centrifuge Setup** area, set the parameters for a centrifugation cycle:

| Parameter | Description |
|--|--|
| Relative Centrifugal Force (0.1–1006.2 x g) | The rotor speed, in RPM (revolutions per minute) and as a multiple of gravity. Default: 251 |
| Acceleration (%) | The rate of centrifugation, as a percent of maximum acceleration. Default: 80 |
| Braking (%) | The deceleration of the centrifuge, as a percent of maximum deceleration. Default: 80 |

| Parameter | Description |
|---------------------------|--|
| Timer Mode | How the specified spin time is implemented: <ul style="list-style-type: none">• Total time. The specified spin time includes acceleration and braking.• Time at speed. The specified spin time does not include acceleration and braking. |
| Time to Spin (sec) | The length of time (seconds) to spin the microplates in the desired mode. Default: 10 |
| Counterweight Mode | The counterweight method used. Select one of the following: <ul style="list-style-type: none">• Fixed counterweight. Ensure that the counterweight is in the centrifuge bucket. The counterweight remains in the bucket during the entire run. For instructions, see the Microplate Centrifuge with Loader User Guide. IMPORTANT For this option, you must remember to place the counterweight in the device before the run and remove it after the run is finished.• Use 2 protocol plates. During the run, the robot places the first two microplates into the centrifuge buckets. After spinning, the robot replaces the two microplates with the next pair of microplates, and so on. IMPORTANT The Use 2 protocol plates counterweight mode requires an even number of microplates. |

Setting up the labware and specifying the run settings

To set up the labware and specify the run settings:

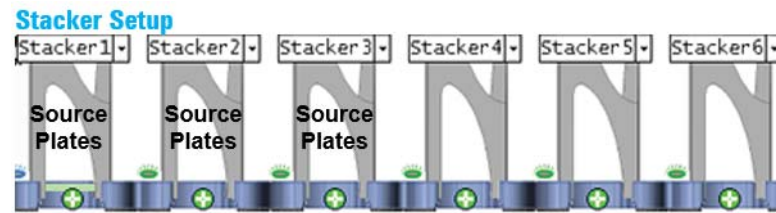
- 1 Set up the Centrifuge for the counterweight mode that you are using:
 - **Fixed counterweight.** Place the counterweight on the Centrifuge Loader stage, and use Centrifuge Loader Diagnostics to move the counterweight into the Centrifuge bucket. The counterweight remains in the Centrifuge bucket during the entire run.
For details on Centrifuge Loader Diagnostics, see the [Microplate Centrifuge with Loader User Guide](#).
 - **Use 2 protocol plates.** Ensure that both Centrifuge buckets are empty.

2 Set up the BenchCel stackers as follows:

- a In the **Stacker Setup** area of the form, determine which stackers are designated for Source plates.

For example, the protocol for the BenchCel 6R designates the first three stackers for source plates. In this case, if you are using fewer than three stackers of labware, fill Stacker 1 before you fill Stacker 2, and so forth.

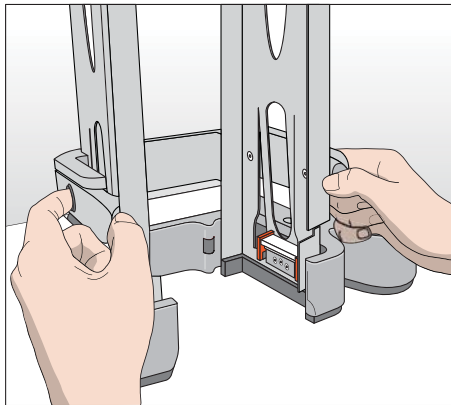
Figure Stacker Setup area for the BenchCel 6R



- b Fill the labware racks on the corresponding BenchCel stackers with the Source plates. For instructions, 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 labware racks.

Figure Opening a labware rack, front-load



3 Locate the **Run Setup** area of the form, and then specify the following:

- a **Number of Plates to Process.** Type the number of Source plates to be centrifuged.
- b **Labware Type.** Select the labware that matches the Source plates in the stacker on the BenchCel device.

4 Confirm that the physical layout of the hardware and labware matches the selections in the form.

CAUTION Improperly seated labware can cause a hardware collision, resulting in equipment damage. Ensure that all labware are properly seated within the stacker.

Running the protocol

Before you start the run



WARNING The BenchCel Workstation has many moving parts that can cause possible pinching, piercing, or bruising. Keep away from the workstation while it is in motion.

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

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:

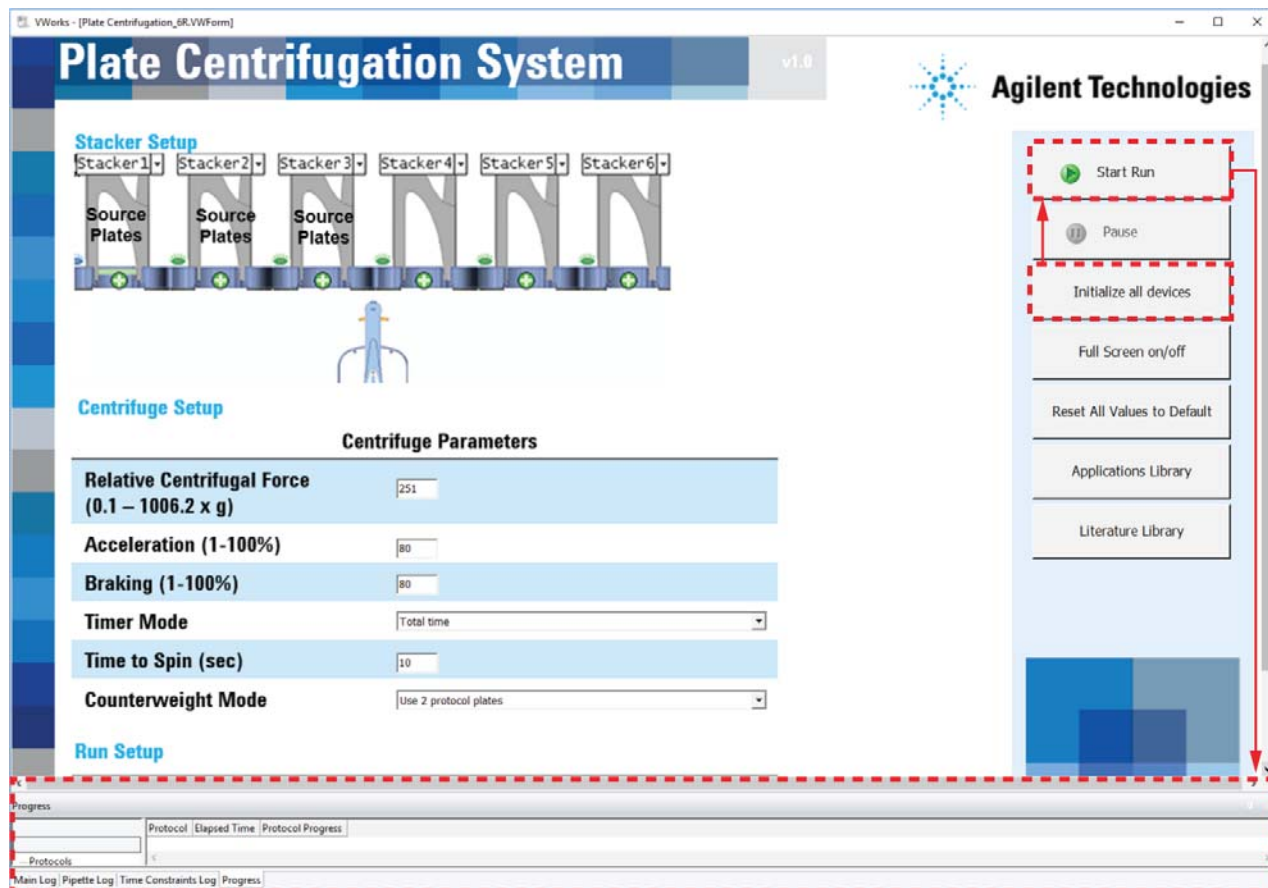
- 1 *Optional.* In the form, click **Initialize all devices**. If you skip this step now, the software will prompt you to initialize the devices in the next step.

The initialization process establishes communication with the BenchCel Microplate Handler and the Microplate Centrifuge with Loader and homes the devices.

- 2 Click **Start Run** .

If you have not already initialized the devices, a message appears asking you to initialize the devices.

The protocol run starts. 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:

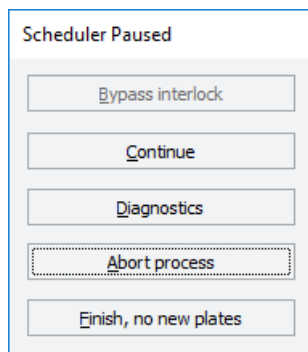
- Click **Yes** to save the parameter settings in the form.
- Click **No** to keep the previously saved parameter settings in the form.

Pausing and continuing the run

To pause and continue the run:

- 1 In the **Plate Centrifugation** 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.

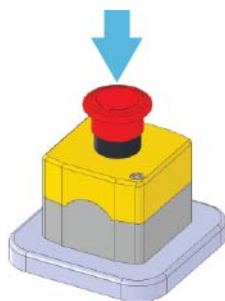
For details about the other options in the Scheduler Paused dialog box, see the [VWorks Automation Control User Guide](#).

Stopping in an emergency

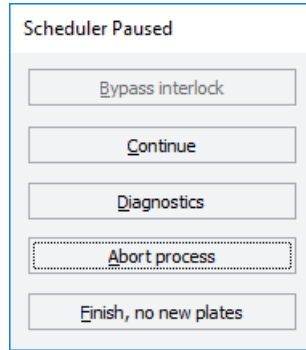
CAUTION You might not be able to resume a protocol after an emergency stop. Do not use an emergency stop to pause a run. To pause and continue a run, use the Pause button.

To stop in an emergency:

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



The Scheduler Paused dialog box opens.



- 2** To reactivate the emergency-stop, turn the red button clockwise. The spring-loaded button pops up.



- 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.



Protocol Guide

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