

Bravo Metabolomics Workbench

Reconstitution

Application Guide

For Research Use Only. Not for use in diagnostic procedures.



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About this guide

Overview

This guide describes the Reconstitution application in the Bravo Metabolomics Sample Prep Platform. For more details on the Bravo Metabolomics Sample Prep Platform, see the [Getting Started Guide](#) in the Literature Library of the Bravo Metabolomics Workbench.

The procedures in this guide assume that you are trained in the safe operation of the Bravo Platform.

WARNING

Using controls, making adjustments, or performing procedures other than those specified in the user documentation can expose you to moving-parts hazards and hazardous voltage. Before using the Bravo Platform, make sure you are aware of the potential hazards and understand how to avoid being exposed to them.

Software version

This guide documents the following versions or later:

- Bravo Metabolomics Workbench 1.0
- VWorks Automation Control 13.1.3
- Bravo Diagnostics 19.1

Related guides

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.
- [G5562A, G5563A Bravo Platform Safety and Installation Guide](#). Describes potential safety hazards and how to avoid them, how to install the Bravo Platform, and how to install the Light Curtain and shields. A copy of this safety guide is included with your shipment.
- [Bravo Platform User Guide](#). Explains how to set up, operate, and maintain the Bravo Platform and how to install accessories.

You can find the workbench user guides in the Literature Library of the Bravo Metabolomics Workbench.

Contacting Agilent Technologies

Web: <https://www.agilent.com>

Contact page: <https://www.agilent.com/en/contact-us/page>

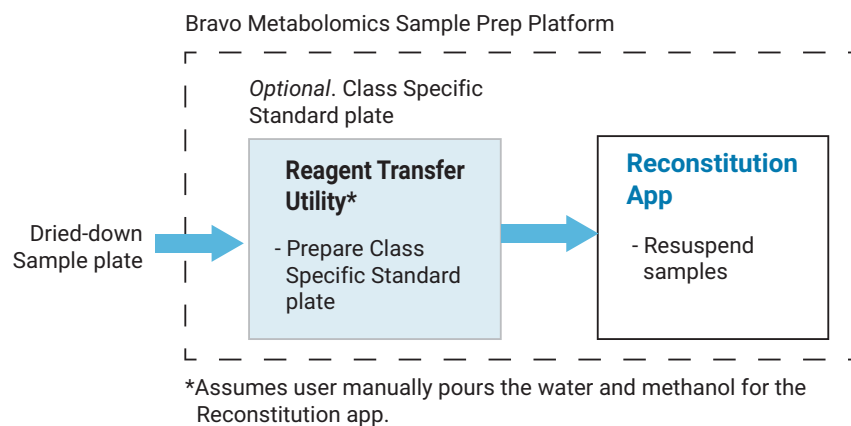
Documentation feedback: documentation.automation@agilent.com

App description

You use the Reconstitution application to re-suspend the contents in a Sample plate that was prepared during a preceding run of the On-Site or Off-Site Plasma Metabolite Extraction application. The Reconstitution application automatically re-suspends the contents of up to 96 wells in the Sample plate as follows:

- 1 Adds Methanol to the Sample plate and then shakes it.
 - 2 Adds water to the Sample plate and then shakes it.
 - 3 *Optional.* Adds a class-specific standard to the Sample plate and then shakes it.
- To prepare the Class Specific Standard plate, you use the Reagent Transfer utility as the following figure shows.

Figure Example of workflow for full plates

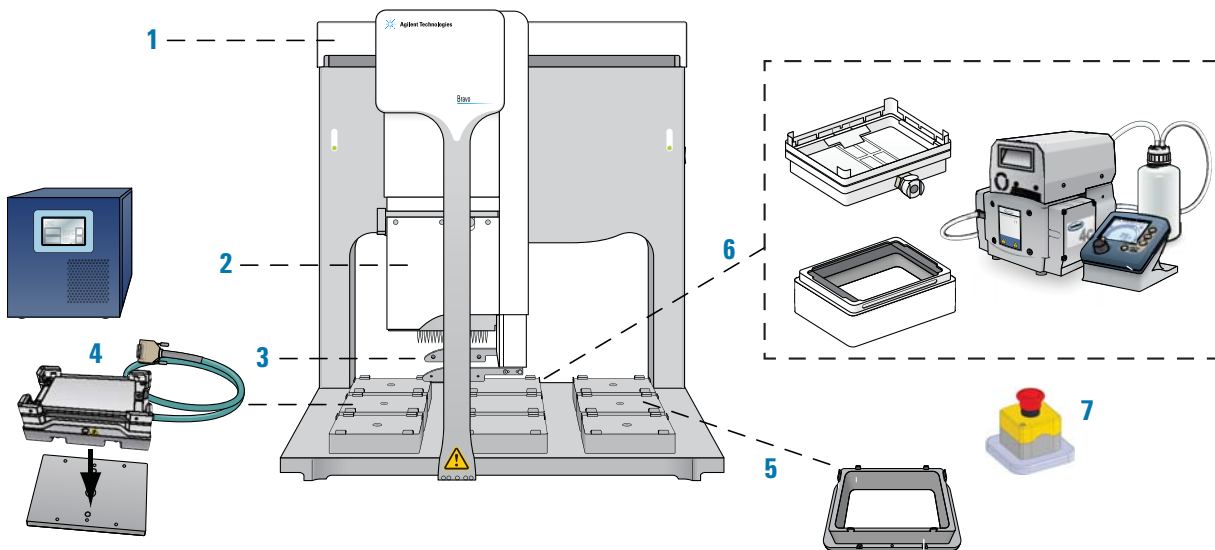


Before you start

Hardware requirements

The following figure and table show the primary hardware components for the Bravo Metabolomics Sample Prep Platform.

Figure Bravo Metabolomics Sample Prep Platform hardware components



Item	Name
1	Bravo Platform
2	Liquid-handling head, 96LT
3	Gripper upgrade
4	Heating Shaking Station (deck location 4) and STC controller
5	Filter Plate Holder (deck location 6)
6	Vacuum Filtration Station and Agilent ME4C NT VARIO Pump <ul style="list-style-type: none"> • Manifold base (deck location 2) • Deep-well collar with black gasket on top (deck location 3)
7	Emergency-stop pendant Light Curtain and safety shields (not shown)

Labware requirements

The Reconstitution protocol requires the following labware:

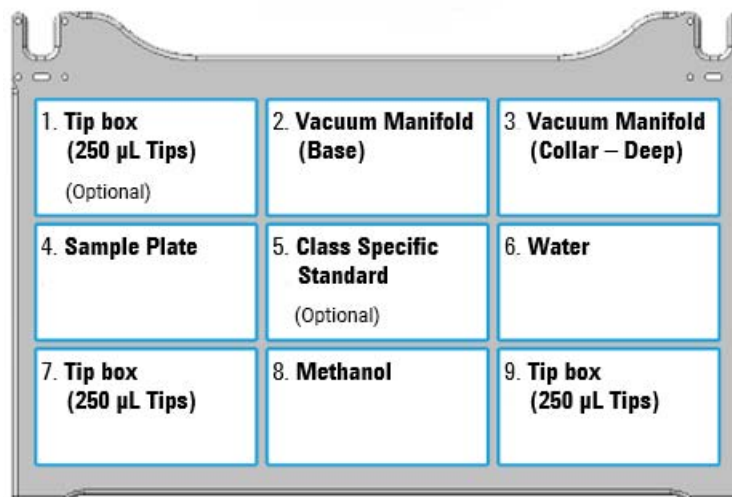
- Tip boxes of Agilent 96 250- μ L pipette tips
At a minimum, 2 tip boxes are required. If you select the optional addition of a class-specific standard, you need 3 tip boxes.
- Sample plate
- If applicable, Class Specific Standard plate
- Water reservoir
- Methanol reservoir

The following figure shows the nine Bravo deck locations for labware. The following table lists the approved labware options for each deck location.

CAUTION

Using a labware type at a deck location other than an approved labware option can cause a collision resulting in equipment damage. Ensure that you use only an approved labware option for each deck location.

Figure Application labware locations on the Bravo deck (top view)



Note: The Class Specific Standard plate (deck location 5) and the tip box at deck location 1 are for the optional addition of a class-specific standard. If you do not select the Class Specific Standard Addition option, this labware is not required.

Table Labware options by Bravo deck location

Deck location	Labware options	Manufacturer part number
1, 7, 9	Tip boxes of 250 µL tips	Agilent 19477.002
4, 5	96 Greiner 655101 PS Clear Round Well Flat Bottom	Greiner 655101
4, 5	96 Costar 3961, PP 2ml assay block	Corning Costar 3961
4, 5	96 EK 2460 PP Rnd Well U Btm	E&K Scientific EK- 2460
4, 5	96 Agilent 203426-100 PP, 1 mL Rnd Btm	Agilent 203426-100
4, 5	96 Agilent A696001000 Captiva collection plate	Agilent A696001000
5, 6, 8	Reservoir, Seahorse 201254-100, PP, no walls, pyramid bottom	Agilent 201254-100
5, 6, 8	96 V11 Manual Fill Reservoir	Agilent G5498B#049

Note: You can use the Tip Transfer utility and Reagent Transfer utility to set up your labware.

Samples and reagents

- Sample plate previously prepared using the On-site or Off-site Plasma Metabolite Extraction application
- Methanol
- Water
- *Optional.* Class-specific standard

You should define which specific standard to use based on your experimental design.

Preparing the sample and reagent labware

IMPORTANT

To prevent evaporation, dispense the reagents into the labware immediately before running the protocol.

CAUTION

Using a labware type at a deck location other than an approved labware option can cause a collision resulting in equipment damage. Ensure that you use only an approved labware option for each deck location.

See “Labware requirements” on page 5. Ensure that the labware is prepared as follows:

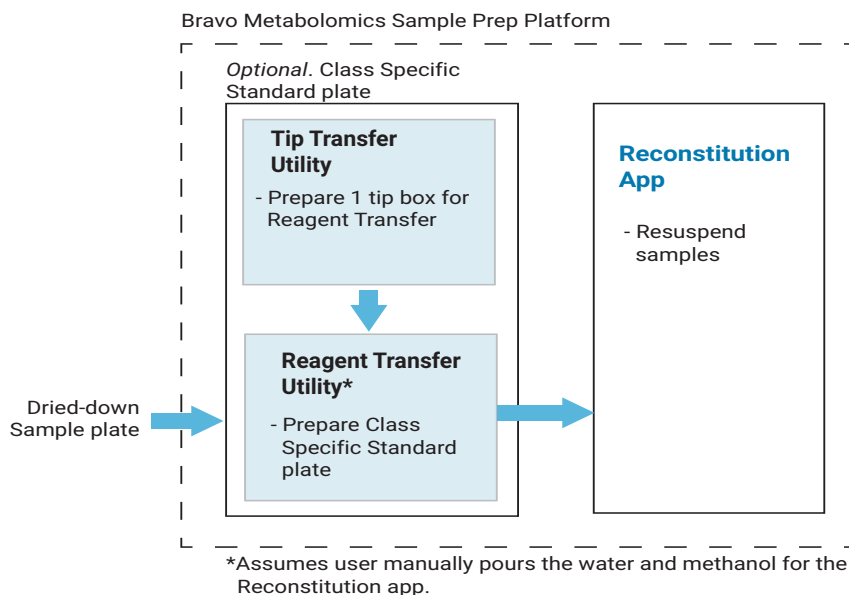
- Fluids are arranged in full columns that are contiguous.
- Tip boxes containing sufficient pipette tips for your run:
 - Tip box for Methanol
 - Tip box for Water
 - If applicable, tip box for optional Class Specific Standard Addition

The columns of tips must start with column 1 in each tip box.

You can use the workbench Reagent Transfer utility to transfer the fluids from one labware to another.

You can use the Tip Transfer utility in the workbench to arrange the pipette tips in the tip boxes.

Figure Example of partial-plate workflow for Reconstitution application

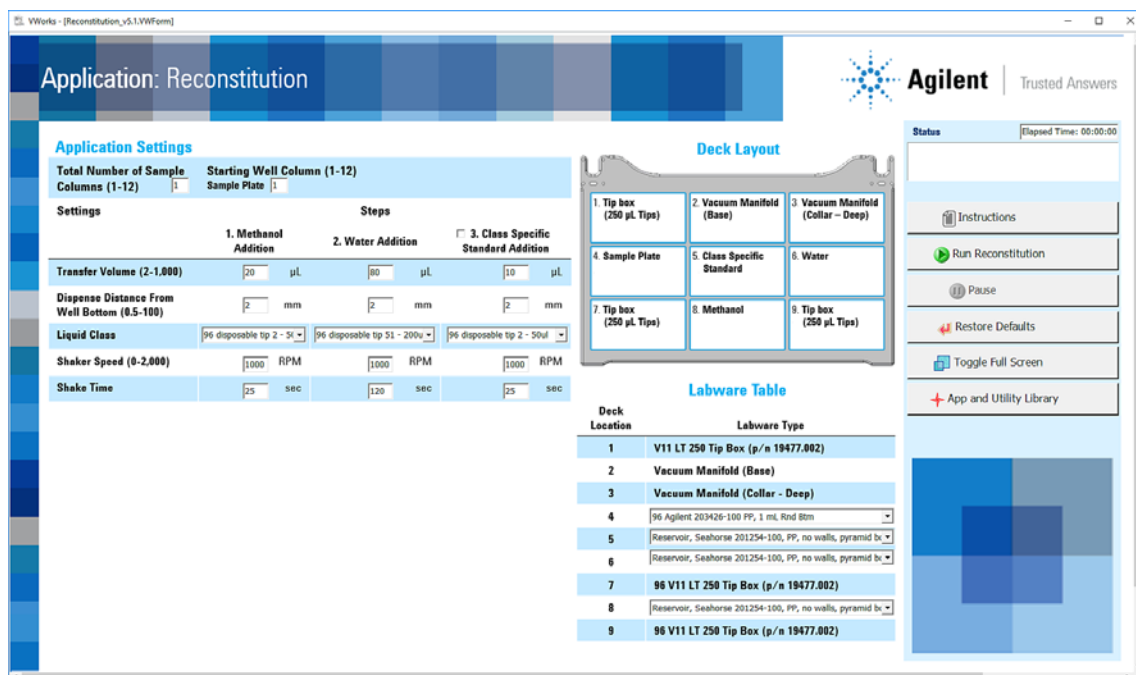
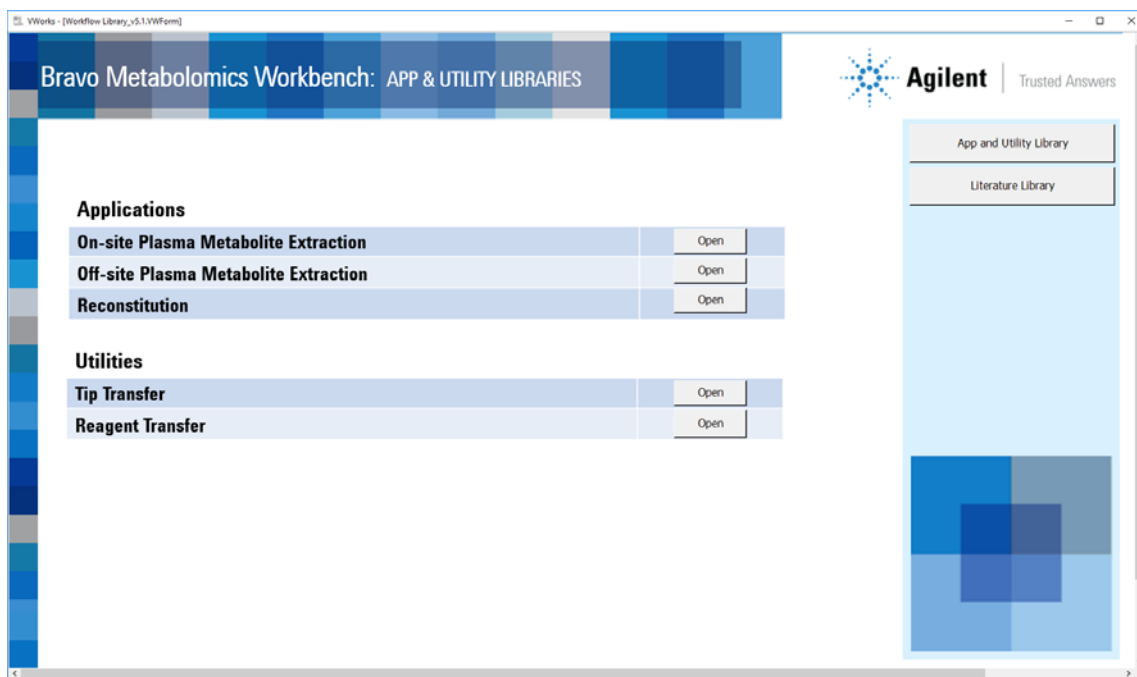


Setting up the protocol

Opening the application

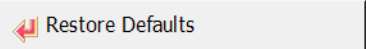
To open the application:

In the **App & Utility Libraries** page of the Bravo Metabolomics Workbench, locate **Reconstitution**, and then click **Open**. The Reconstitution form opens.



Specifying the settings

To specify the settings:

- Optional. Click  to set all Application Settings to their defaults.
- Under **Application Settings**, specify the following:

Total Number of Sample Columns (1-12)	<input type="text" value="1"/>	Starting Well Column (1-12) Sample Plate	<input type="text" value="1"/>
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Setting	Description
Total Number of Sample Columns (1–12)	The number of columns of sample wells in the Sample plate at deck location 4, for example, type 12 for a full plate.
Starting Well Column (1–12)	
Sample Plate	The starting column of destination wells in the Sample plate at deck location 4. For a full-plate run, this is column 1.

- Specify the **Settings** for the following **Steps**:
 - 1. Methanol Addition
 - 2. Water Addition
 - Optional. 3. Class Specific Standard Addition
 Select the check box if you want to include the standard. Otherwise, clear the check box.

	1. Methanol Addition	2. Water Addition	<input checked="" type="checkbox"/> 3. Class Specific Standard Addition
Transfer Volume (0-1,000)	<input type="text" value="20"/> μL	<input type="text" value="80"/> μL	<input type="text" value="10"/> μL
Dispense Distance From Well Bottom (0-100)	<input type="text" value="5"/> mm	<input type="text" value="5"/> mm	<input type="text" value="5"/> mm
Liquid Class	<input type="text" value="96 disposable tip 2 - 5("/>		

The following table describes the settings for these steps.

Table Settings for Reconstitution Steps 1 to 3

Setting	Description
Transfer Volume	<p>The volume of liquid per pipette tip to transfer from the reservoir or plate to the Sample plate (deck location 4).</p> <p>If this volume setting is greater than the capacity of the pipette tips, the transfer will be evenly split into multiple aspirate-and-dispense cycles.</p> <p>Defaults:</p> <ol style="list-style-type: none">1 Methanol Addition 20 (µL)2 Water Addition 80 (µL)3 Class Specific Standard Addition 10 (µL) <p>Range: 2–500 (µL)</p>
Dispense Distance From Well Bottom	<p>The dispense distance between the end of the pipette tips and the well bottoms during the liquid transfer.</p> <p>The labware definition must be accurate and the teachpoint must be precise in order for the system to position the tips at the correct distance from the well bottom.</p> <p>Default: 2 (mm)</p> <p>Range: 0.5–100 (mm)</p>
Liquid Class	<p>The pipetting speed and accuracy for the liquid transfer. You may choose from the options, which are based on the tip type and volume being transferred. These are good general-purpose liquid classes for most reagents:</p> <ul style="list-style-type: none">• 96 disposable tip 1 -2 µL• 96 disposable tip 2 - 50 µL• 96 disposable tip 51 - 200 µL
Shaker Speed	<p>The shake speed, in revolutions per minute (RPM) used to shake the Sample plate (deck location 4) on the Shaking Station.</p> <p>Default: 600 (RPM)</p> <p>Range: 0–2000 (RPM)</p>
Shake Time	<p>The time (seconds) used to shake the Sample plate on the Shaking Station.</p> <p>Defaults:</p> <ol style="list-style-type: none">1 Methanol Addition 25 (s)2 Water Addition 120 (s)3 Class Specific Standard Addition 25 (s) <p>Range: 0–86,400 (s) or (0–24 hours)</p>

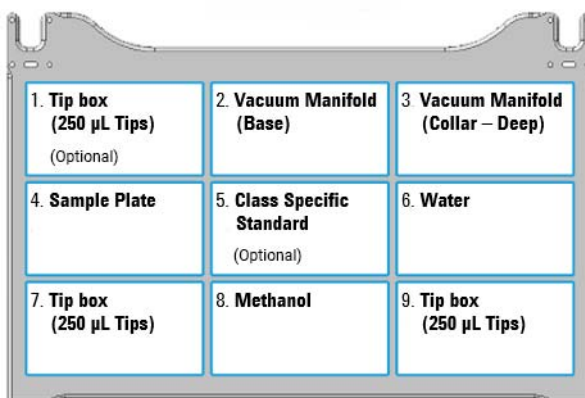
Specifying the labware

CAUTION

Using a labware type at a deck location other than an approved labware option can cause a collision resulting in equipment damage. Ensure that you use only an approved labware option for each deck location.

To specify the labware:

- 1 In the form, refer to the **Deck Layout**.



- 2 In the **Labware Table**, select the labware you are using for deck locations 4, 6 and 8. If you are adding a class specific standard, select the labware for deck location 5.

Deck Location	Labware Type
1	V11 LT 250 Tip Box (p/n 19477.002)
2	Vacuum Manifold (Base)
3	Vacuum Manifold (Collar - Deep)
4	96 Agilent 203426-100 PP, 1 mL Rnd Btm
5	Reservoir, Seahorse 201254-100, PP, no walls, pyramid b
6	Reservoir, Seahorse 201254-100, PP, no walls, pyramid b
7	96 V11 LT 250 Tip Box (p/n 19477.002)
8	Reservoir, Seahorse 201254-100, PP, no walls, pyramid b
9	96 V11 LT 250 Tip Box (p/n 19477.002)

Running the protocol

Before you start

- Prepare the sample and reagents. See [“Preparing the sample and reagent labware” on page 7](#).
- Ensure that you have tip boxes of fresh pipette tips containing the required tips for your run:
 - **Full-plate run.** Use three full tip boxes if the optional class-specific standard is included in your method. Otherwise, use two full tip boxes.
 - **Partial-plate run.** Use partially filled tip boxes. Ensure that the pipette tips are arranged in full, contiguous columns starting at column 1. The number of columns should correspond to the number of columns in the Sample plate. You can use the workbench Tip Transfer utility to set up the tip boxes.

WARNING

If you touch any of the moving parts or attempt to move labware while the Bravo Platform is in operation, the device could pinch, pierce, or bruise you. Keep your fingers, hair, clothing, and jewelry away from the device while it is in motion.

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 using empty 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. To decrease the run time, you can decrease the volumes and shake time.


Starting the protocol run

To start the protocol run:

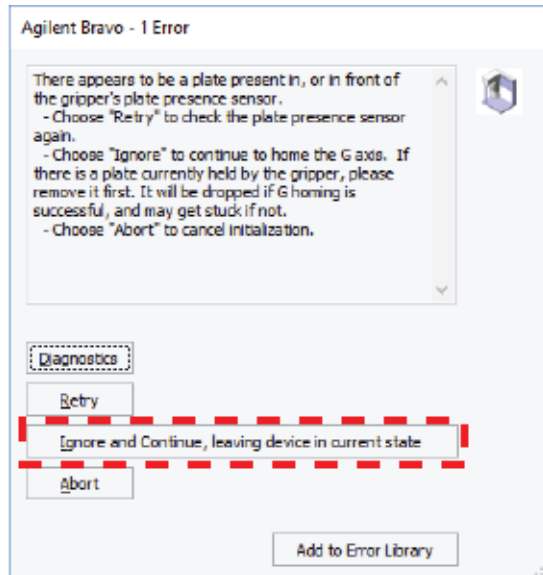
- 1 Review the selections in the protocol form to confirm they are correct.
- 2 Verify that the physical layout on the Bravo deck matches the **Deck Layout** image in the form. Make sure the labware are properly seated within the platepads on the Bravo deck.

CAUTION

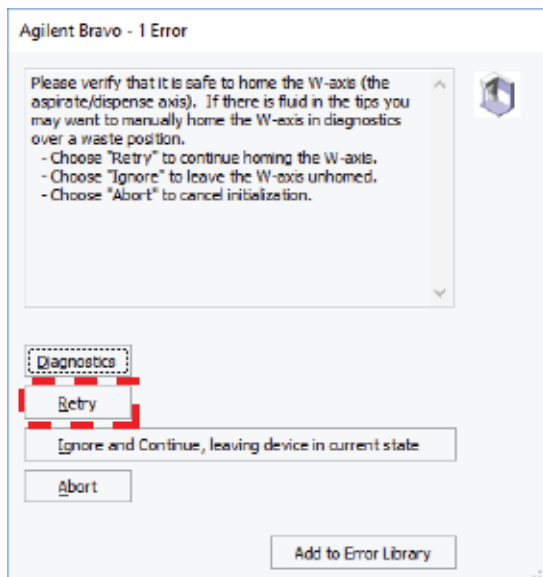
Improperly seated labware can cause a hardware collision, resulting in equipment damage. Ensure that all labware are properly seated within the alignment features of their respective platepads.

- 3 Click .
 - If this is the first time the utility has been run after powering up the Bravo Platform, the device initialization process begins. Proceed to step 4.
 - If the platform is already initialized, skip to step 6.

- 4 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, leaving device in current state** to continue the initialization.



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

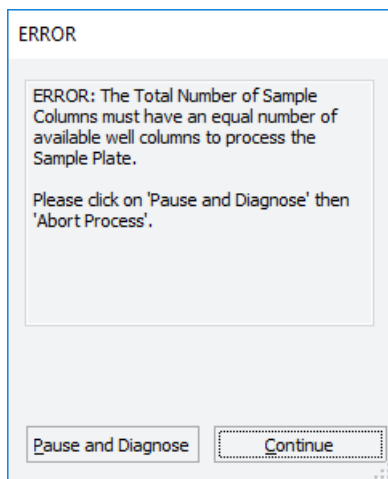


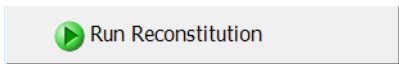
When the initialization process is finished, the orange lights on the Bravo Platform light panel flicker briefly and then begin to flash.

- 6 When the **Bravo Deck Setup** dialog box appears, verify that the deck layout is correct.
 - If it is correct, click **Continue**.
 - If it is not correct, click **Pause and Diagnose**, and then click **Abort process** in the Scheduler Paused dialog box. Resolve the problem, and then restart the run.

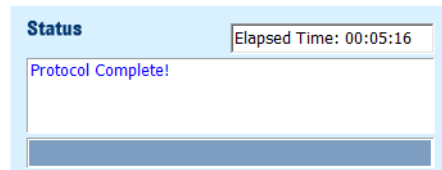


- 7 If an Error message appears, the software detects a conflict in the protocol setup.
 - a Follow the on-screen instructions to click **Pause and Diagnose**, and then click **Abort process** in the Scheduler Paused dialog box.
 - b Resolve the conflict described in the error message, for example:



- Insufficient number of columns of pipette tips
 - Unequal number of columns selected for source and destination plates
- c Click  to restart the run.

To monitor the progress of the run, check the **Status** area of the form.



About stopping or pausing a run

IMPORTANT

Attempting to pause a running protocol to change a setting can be detrimental to the protocol. If you need to change a setting in a protocol that is actively running, pause the protocol, select Abort process from the Scheduler Paused dialog box, change the setting, and then restart the protocol.

For more detailed instruction, see the [Bravo Metabolomics Sample Prep Platform Getting Started Guide](#).

Cleaning up

When the protocol run is finished, make sure you:

- Remove all labware from the Bravo deck.
- Discard solutions, organic waste, and used labware following appropriate waste disposal procedures.

WARNING

Make sure you discard the chemical waste and used labware according to your lab's waste disposal procedures and in compliance with all local, state, and federal safety regulations.

Automation movements during the protocol

This section describes the basic movements of the Bravo Platform during the protocol using the default protocol settings. Changing the selections or parameters will alter the movements.

Protocol step	Head moves to deck location...	Action
Tip Pick Up for Methanol Transfer	7	Presses on the columns of pipette tips from the tip box. The number of full columns corresponds to the specified number of selected sample columns.
Methanol Addition	8, 4	Transfers the liquid from the Methanol reservoir into the specified columns of wells in the Sample plate on the Shaking Station. The transfer cycle may repeat depending on the volume to be transferred.
Shaking and Mixing	4	Shakes the plate at the specified speed and performs the selected number of mix cycles.
Tip Change	7	Ejects the used Methanol pipette tips into the tip box at deck location 7.
	9	Presses on the columns of fresh pipette tips for the water addition. The number of full columns corresponds to the specified number of selected sample columns.
Water Addition	6, 4	Transfers the liquid from the water reservoir into the specified columns of wells in the Sample plate on the Shaking Station. The transfer cycle may repeat depending on the volume to be transferred.
Shaking and Mixing	4	Shakes the plate at the specified speed and performs the selected number of mix cycles.
Tip Removal	9	Ejects the used pipette tips from the water transfer into the tip box at deck location 9.
Optional Standard Addition	1	Presses on the columns of fresh pipette tips for the standard addition. The number of full columns corresponds to the specified number of selected sample columns.
	5,4	Transfers the class-specific standard from the plate at deck location 5 into the specified columns of wells in the Sample plate on the Shaking Station. The transfer cycle may repeat depending on the volume to be transferred.
	4	Shakes the plate at the specified speed and performs the selected number of mix cycles.
	1	Ejects the used pipette tips from the standard transfer into the tip box at deck location 1.