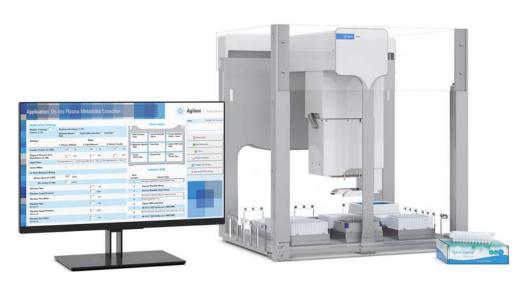


# Bravo Metabolomics Sample Prep Platform

# **Getting Started Guide**

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



This guide contains the following topics:

- "About this guide" on page 2
- "Hardware components" on page 3
- "Bravo Metabolomics Workbench overview" on page 6
- "Workflow overview" on page 11
- "Starting up and shutting down" on page 14
- "Accessing an application or utility" on page 17
- "About simulating a protocol run" on page 18
- "Stopping or pausing a run" on page 19
- "Labware requirements" on page 21
- "About the installed files" on page 25
- "Reporting problems" on page 26

# About this guide

This guide describes the Bravo Metabolomics Sample Prep Platform and how to access the applications and utilities. This guide assumes that the Bravo Metabolomics Sample Prep Platform has been installed properly and that you are trained in the safe operation of the Bravo Platform.



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 requirements

The Bravo Metabolomics Sample Prep Platform requires the following software:

- Microsoft Windows 10 64-bit operating system
- VWorks Automation Control software 13.1.3
- Bravo Metabolomics Workbench 1.0

### Related guides and where to find them

For a list of the workbench user guides, see "Applications" on page 8 and "Utilities" on page 9.

In addition, 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.
- G5562A, G5563A Bravo Platform Safety and Installation Guide. Describes how to avoid potential safety hazards on the Bravo Platform and how to install the instrument, Light Curtain, and shields.
- Bravo Platform User Guide. Explains how to set up, operate, and maintain the Bravo Platform and how to install accessories.
- VWorks Automation Control Setup Guide. Explains how to define labware and labware classes, liquid classes, and pipetting techniques, and how to manage user accounts.

#### To access the user guides for Automation Solutions products, do one of the following:

- From within VWorks software, select **Help** > **Knowledge Base** or press F1.
- From the Windows desktop, select Start > Agilent Technologies > VWorks Knowledge Base.
- Go to the online VWorks Knowledge Base at www.agilent.com/chem/askb.

### **Contacting Agilent Technologies**

Web: https://www.agilent.com

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

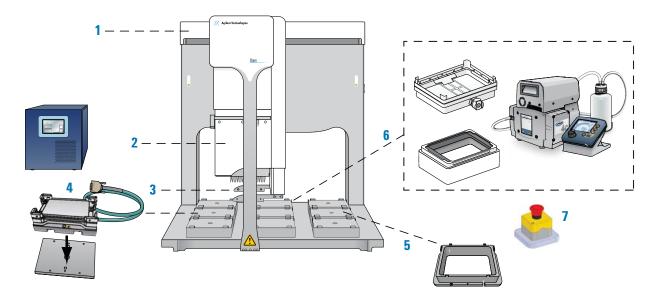
Documentation feedback: documentation.automation@agilent.com

# Hardware components

## **Primary components**

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

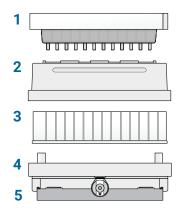


Item	Name			
1	Bravo Platform			
2	Liquid-handling head, 96LT			
3	Gripper upgrade			
4	Heating Shaking Station (deck location 4) and STC controller			
5	Filter Plate Holder (deck location 6)			
6	<ul> <li>Vacuum Filtration Station and Agilent ME4C NT VARIO Pump</li> <li>Manifold base (deck location 2)</li> <li>Deep-well collar with black gasket on top (deck location 3)</li> </ul>			
7	Emergency-stop pendant Light Curtain and safety shields (not shown)			

### **Vacuum Filtration Station**

The Vacuum Filtration Station is used to remove lipids from the plasma sample. The following figure shows the labware stacking configuration on the Vacuum Filtration Station, and the table describes each component.

Figure Labware stacking configuration (side view)



Item	Name	Description	
1	Captiva EMR-Lipid plate	The Captiva EMR-Lipid, 1 mL, 96-well plate (Agilent 5190-1001) provides highly selective and efficient lipid removal without unwanted metabolite loss.	
2	Deep-well collar with black gaskets	The deep-well collar is designed to accommodate the use of 2 mL deep-well collection plates. The collar has two black gaskets held in place by adhesive. One black gasket adheres to the top of the collar to accommodate the Captiva EMR-Lipid plate. The second black gasket adheres to the underside surface of the collar top to maintain the correct spacing underneath the collar.  Note: The collar should be seated on the platepad at Bravo deck location 3 before starting a Plasma Metabolite Extraction application.	
3	Collection plate	A 2-mL deep-well plate collects the eluate from the filtration process. See "Labware requirements" on page 21 for a listing of the deck location 1 options for the Plasma Metabolite Extraction applications.	

Item	Name	Description			
4	Base, manifold	The vacuum base is installed on the Bravo at deck location 2.			
		The base manifold connects to the vacuum source, which is the Agilent ME4C NT VARIO Pump.			
5	Short platepad	The manifold base is installed on a short platepad to compensate for the fully assembled height of the station including the labware.			

# IMPORTANT

If the Vacuum Filtration Station configuration is too tall for the Bravo gripper to assemble without the risk of a collision, an error will occur during the run. Ensure that the vacuum manifold base is installed on a short platepad (SRT platepad).

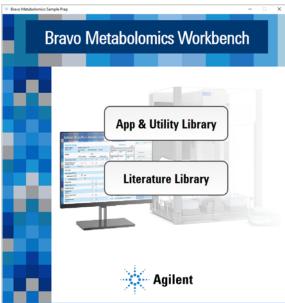
During the Plasma Metabolite Extraction applications, the Bravo Metabolomics Sample Prep Platform automatically assembles the Vacuum Filtration Station as follows:

- 1 Moves the Collection plate (deck location 1) to the manifold base (deck location 2).
- 2 Moves the deep-well collar onto the Collection plate on the manifold base (deck location 2).
- **3** Moves the Captiva EMR-Lipid plate from the Filter Plate Holder (deck location 6) and places it atop the deep-well collar (deck location 2).

# Bravo Metabolomics Workbench overview

The Bravo Metabolomics Workbench software is a form-based user interface for the VWorks software.

**Figure** Bravo Metabolomics Workbench window



The Bravo Metabolomics Workbench software includes the following:

- App and Utility Library. The App and Utility Libraries page provides links to the applications and utilities in the workbench. See "About the App & Utility Libraries" on page 7.
- **Literature Library**. The Literature Library page provides links to the user guides for the Bravo Metabolomics Sample Prep Platform. For details, see "About the Literature Library" on page 9.

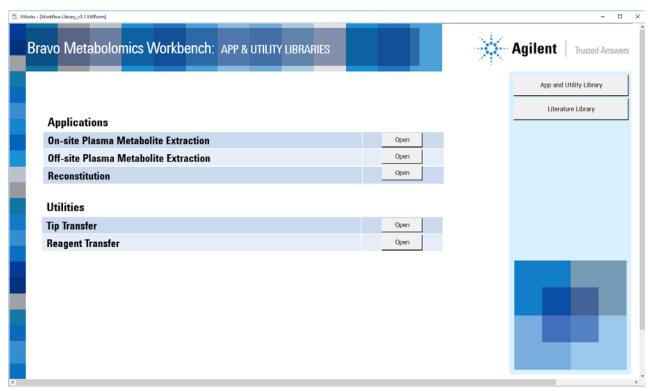
The software includes the VWorks device file and profile for connecting to your Bravo Platform, and the files required to run the protocols. For details on the installed files, see "About the installed files" on page 25.

### About the App & Utility Libraries

The App & Utility Libraries page is a collection of the following:

- **Applications**. Sample prep protocols that can be combined into a workflow. For examples, see "Workflow overview" on page 11.
- **Utilities**. Protocols that automate specific tasks (for example, general liquid handling) to set up labware for an application protocol, transition between applications in a workflow, or transition between an application and loading onto an analytical device.

Figure App & Utility Libraries page



# **Applications**

You use the applications to prepare the metabolomics samples for downstream processing.

Application	Description	See
On-site Plasma Metabolite Extraction	Automatically performs sample preparation on up to 96 plasma samples collected on site. This application does the following:	On-site Plasma Metabolite Extraction Application Guide
	Plasma sample quenching	
	Lipid removal	
	Transfer of the eluate to a new plate to normalize the volumes	
	The final plate can be dried down for storage. Later you can run the Reconstitution application to re-suspend the samples for downstream processing.	
Off-site Plasma Metabolite Extraction	Automatically performs sample preparation on up to 96 plasma samples collected off site (off-deck sample quenching performed at the collection site). This application does the following:	Off-site Plasma Metabolite Extraction Application Guide
	Lipid removal	
	Transfer of the eluate to a new plate to normalize the volumes	
	The final plate can be dried down for storage. Later you can run the Reconstitution application to re-suspend the samples for downstream processing.	
Reconstitution	Automatically re-suspends the contents of up to 96 wells in a sample plate for downstream processing.	Reconstitution Application Guide

### **Utilities**

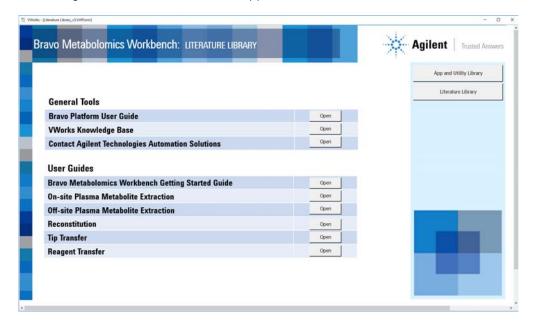
You use the utilities to prepare the labware for the Metabolomics sample prepapplications.

Utility	Description	See
Tip Transfer	Automatically moves full columns of disposable pipette tips from a source tip box to a destination tip box. For example, you can use the utility to prepare the tip boxes for a partial-plate run.	Bravo Metabolomics Workbench Tip Transfer Utility Guide
Reagent Transfer	Automatically transfers 1–12 columns of reagents or samples in parallel from a source plate or reservoir into a destination plate.	Bravo Metabolomics Workbench Reagent Transfer Utility Guide

### **About the Literature Library**

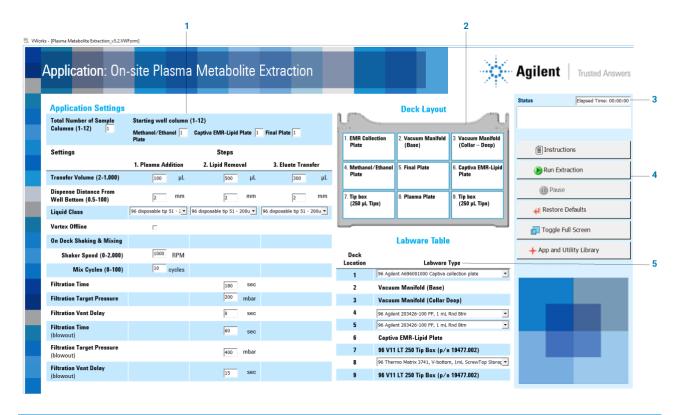
The Literature Library provides access to the following:

- Bravo Platform User Guide
- VWorks Knowledge Base
- User guides for the metabolomics applications and utilities



## Application and utility form components

This section describes the primary components of the forms in the Bravo Metabolomics Workbench.



Item	Name	Description	
1 Application Settings Specifies the parameters for the protocol run.		Specifies the parameters for the protocol run.	
2 Deck Layout		Displays the labware layout on the Bravo deck for the protocol run.	
3	Status	Displays the progress of a protocol that is currently running.	

Item	Name	Description			
4	Navigation buttons				
	Instructions	Displays the pdf of the corresponding application or utility guide.			
	Run <app type=""></app>	Starts the protocol run of the corresponding application or utility, for example, Run Extraction, Run Reconstitution, or Run Transfer.			
	Pause	Pauses the run and displays the Scheduler Paused dialog box. For details, see "Stopping or pausing a run" on page 19.			
	Restore Defaults	Restores the parameter values to the default settings.			
		Changes how the form displays in the VWorks window:			
	Screen	Full Screen on. The form completely fills the VWorks window.			
		<ul> <li>Full Screen off. The form is partially displayed within the VWorks window, enabling access to the VWorks menus, toolbars, and logs.</li> </ul>			
access the toolbar to change the a protocol run" on page 18.		For example, if you want to perform a simulated protocol run, you need to access the toolbar to change the simulation mode. See "About simulating a protocol run" on page 18.			
		Returns the display to the App & Utility Libraries page.			
5	Labware Table	Specifies the labware by deck location for the given protocol.			

# Workflow overview

## **Bravo Metabolomics Workbench workflow**

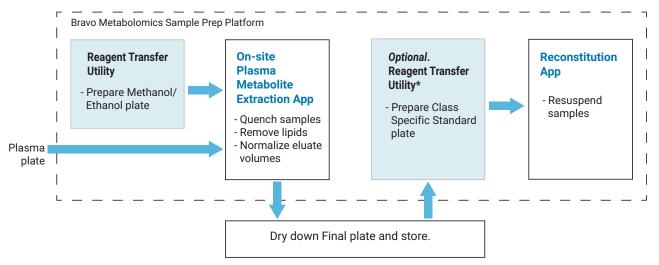
Step	For this task	See
1	Start up the Bravo Metabolomics Sample Prep Platform.	"Starting up and shutting down" on page 14
2 Open the application or utility you want to "Accessing an a run.		"Accessing an application or utility" on page 17
3	Determine the labware requirements.	"Labware requirements" on page 21
4	Prepare the labware for the application you are running.	Tip Transfer Utility Guide and Reagent Transfer Utility Guide
5	Run the protocol for the On-site or Off-site Plasma Metabolite Extraction application.	On-site Plasma Metabolite Extraction Application Guide
		Off-site Plasma Metabolite Extraction Application Guide
6	Dry down the final plate in a SpeedVac for storage.	_

Step	For this task	See
7	Prepare the labware for the Reconstitution application.	Tip Transfer Utility Guide and Reagent Transfer Utility Guide
8	Re-suspend the samples for downstream processing.	Reconstitution Application Guide

## **Application workflows**

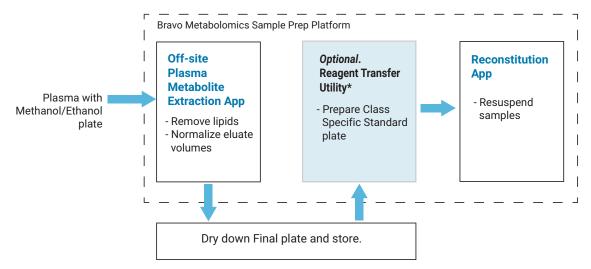
The following figures show the automated sample prep workflows for the On-site and Off-site Plasma Metabolite Extraction applications using full plates.

Figure Example of On-site Plasma Metabolite Extraction using full plates



<sup>\*</sup>Assumes that the user manually pours the methanol and water for the Reconstitution app.

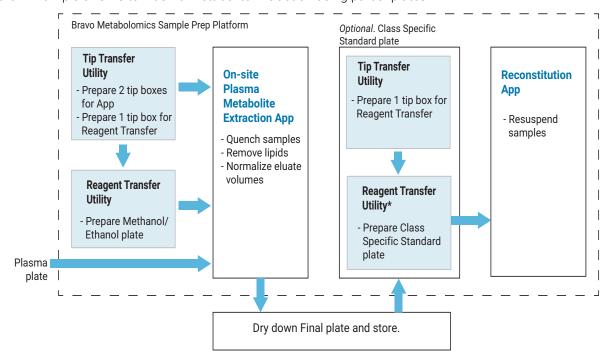
Figure Example of Off-site Plasma Metabolite Extraction using full plates



<sup>\*</sup>Assumes that the user manually pours the methanol and water for the Reconstitution app.

The following example shows a partial-plate workflow for the On-site Plasma Metabolite Extraction application. For partial plates, the tip boxes must contain full, contiguous columns of 8 pipette tips each starting in column 1. You use the Tip Transfer utility to prepare the tip boxes. The fluids must also be arranged in full columns that are contiguous, and you use the Reagent Transfer utility to prepare the corresponding labware.

Figure Example of On-site Plasma Metabolite Extraction using partial plates



<sup>\*</sup>Assumes that the user manually pours the methanol and water for the Reconstitution app.

# Starting up and shutting down



Always turn off the Bravo Platform before installing or uninstalling a liquid-handling head. Failure to do so can damage the head electronics.

### Starting up the Bravo Metabolomics Sample Prep Platform

### To start up the Bravo Metabolomics Sample Prep Platform:

- 1 Ensure that the main power cable and Ethernet are plugged into the Bravo connection panel.
- 2 Turn on the accessories:
  - **a** STC Controller for the Shaking Station





**b** Vacuum Filtration Station pump



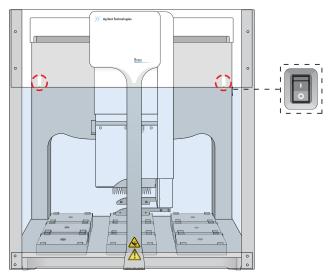
Ensure all the tubing is connected, and, If applicable, empty the waste

- 3 Turn on the computer and the monitor, and start the Microsoft Windows operating system.
- **4** If you have not already done so, install the liquid-handling head. For instructions, see the *Bravo Platform User Guide*.

**5** On the side of the Bravo Platform, press the power switch to the **on (I)** position. The front panel status lights turn blue.

Note: The status lights will turn red if the emergency-stop pendant is pressed or absent or if the Light Curtain is interrupted.

Figure Bravo status lights and power switch



- **6** To start the software:
  - On the Microsoft Windows desktop, click the shortcut for the Bravo
     Metabolomics Workbench
  - Alternatively, in the Windows Apps list, click Agilent Technologies > Bravo Metabolomics Workbench.

The Bravo Metabolomics Workbench window opens.



7 Click App & Utility Library. The VWorks software starts.

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



Note: User accounts should be set up in advance by the system administrator. For details, see the VWorks Automation Control Setup Guide.

### **Establishing communication**

The Bravo Platform must be initialized to establish communication between the software and the hardware. The initialization process starts automatically the first time you run a workbench application or utility protocol after starting up the platform.

If you want to initialize the device before running any protocols, see the *Bravo Platform User Guide* for instructions.

### Shutting down the Bravo Metabolomics Sample Prep Platform

Shut down the Bravo Metabolomics Sample Prep Platform before you:

- Clean the Bravo Platform
- Change the liquid-handling head
- Install accessories
- Move the Bravo Platform

#### To shut down the Bravo Metabolomics Sample Prep Platform:

- 1 If you plan to uninstall the liquid-handling head, ensure that no disposable pipette tips remain on the head.
- **2** Optionally, home the liquid-handling head.
- **3** Shut down the computer.
- 4 Turn off the accessories.
- **5** On the side of the Bravo Platform, press the power switch to the **off (o)** position.
- **6** If applicable, uninstall the liquid-handling head.

# Accessing an application or utility

### To access an application or utility:

1 In the **App & Utility Libraries** page, locate the name of the application or utility you want to use, and then click **Open**.



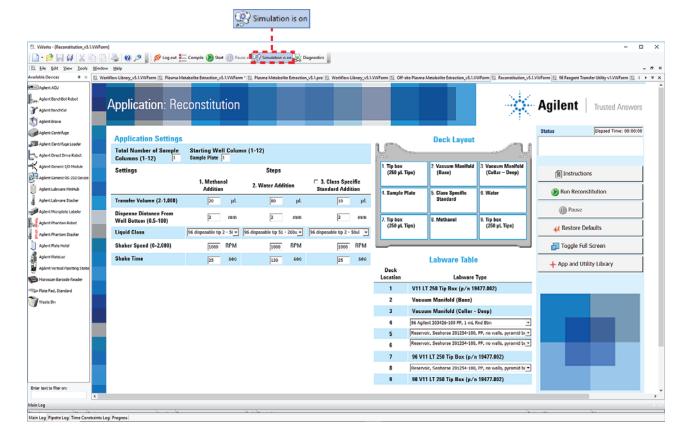
The form for the corresponding application or utility opens in the VWorks window. For a general description of the form components, see "Application and utility form components" on page 10.

# About simulating a protocol run

A protocol simulation is a virtual run where the software performs the tasks without moving robots or labware. For more details on simulated runs, see the *VWorks Automation Control User Guide*.

#### To turn on or off the simulation mode:

- 1 In the Bravo Metabolomics Workbench form, click **Toggle Full Screen** to change the display mode so that the VWorks toolbar is visible.
- **2** Do one of the following:
  - To turn on simulation, click Simulation is off. The button label changes to Simulation is on.
  - To turn off simulation, click Simulation is on. The button label changes to Simulation is off.



# Stopping or pausing a run

### Pausing and continuing a run

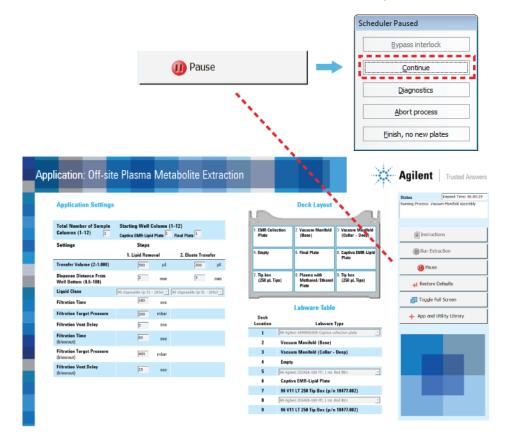
You can pause a run to introduce a labware that was forgotten during the setup, add reagents to a labware that is at risk of depleting, and so forth.



Attempting to pause a running protocol to change a setting can be detrimental to the protocol.

#### To pause and then continue a run:

- 1 In the application or utility control panel, click Pause.
  The task currently in progress finishes before the protocol pauses. The Scheduler Paused dialog box opens.
- While the Bravo device is idle, make the necessary changes to your run, for example, adjusting a labware position or volume.
- **3** To resume the run, click **Continue** in the **Scheduler Paused** dialog box.



#### To abort a run:

- In the application or utility control panel, click Pause.
  The task currently in progress finishes before the protocol pauses. The Scheduler Paused dialog box opens.
- 2 In the Scheduler Paused dialog box, click Abort process.

### **Emergency stops**

An emergency stop might be necessary to prevent a collision of the Bravo head with another object, such as misaligned labware. If you want to temporarily pause and then continue a run, see "Pausing and continuing a run" on page 19.

The Bravo Platform has a safety interlock circuit that is designed to protect operators from moving-parts hazards. The safety interlock circuit must be closed for the Bravo Platform to operate. The pendant and the Light Curtain are connected to the interlock circuit. Pressing the red button on the pendant or interrupting the Light Curtain trips the interlock circuit, causing the motion of the Bravo head to stop.

### To stop in an emergency:

Press the red button on the emergency-stop pendant. The Bravo head stops moving.

Figure Emergency-stop pendant



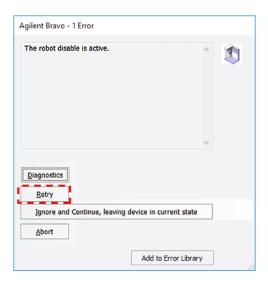
The Bravo Error dialog box opens and displays a robot disable is active error message.

### To restore the Bravo device after an emergency stop:

At the emergency-stop pendant, turn the red button clockwise. The spring-loaded button pops up.

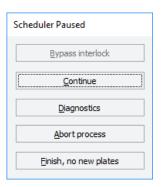


- 2 If applicable, remove any object that is interrupting the Light Curtain.
- In the **Bravo Error** dialog box, click **Retry**. In most cases, the Bravo Platform will be able to resume the run where it left off.



### To abort the run after an emergency stop:

- 1 In the original **Bravo Error** dialog box, click **Abort**.
- 2 In the Scheduler Paused dialog box that opens, click Abort process.



# Labware requirements



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.

The following table lists the default labware settings for the applications and utilities. The rest of this section lists the labware options by Bravo deck location for each application and utility.

### Labware default selections in the applications and utilities

Application or utility	96 Agilent A696001000 Captiva collection plate	Reservoir, Seahorse 201254-100, PP, no walls, pyramid bottom	96 Agilent 203426-100 PP, 1 mL Rnd Btm	96 well Captiva EMR-Lipid Filter plate	96 Thermo 3741, V- bottom, ScrewTop, Storage Tubes	V11 LT250 Tip Box 19477.002
On-site Plasma Metabolite Extraction	1		2	1	1	2
Off-site Plasma Metabolite Extraction	1		2	1	-	2
Reconstitution	_	3	1	_	_	3
Reagent Transfer	_	2	_	_	_	1
Tip Transfer	_		_	_	_	2

## On-site Plasma Metabolite Extraction application labware options

The following table lists the approved labware options for each deck location. Some deck locations are limited to one labware type, while other locations can accommodate several labware types.

Deck location	Labware options	Manufacturer part number
1, 4, 5	96 Costar 3961 PP 2ml assay block	Corning Costar 3961
1, 4, 5	96 Agilent A696001000 Captiva collection plate	Agilent A696001000
4, 5	96 Agilent 203426-100 PP, 1 mL Rnd Btm	Agilent 203426-100
5	96 EK 2460 PP Rnd Well U Btm	E&K Scientific EK- 2460
5	96 Greiner 655101 PS Clr Rnd Well Flat Btm	Greiner 655101
6	96 well Captiva EMR-Lipid Filter plate (Captiva EMR - Lipid plate)	Agilent 5190-1001
7, 9	96 V11 LT Tip Box (250 μL disposable pipette tips)	Agilent 19477.002
8	96 Thermo Matrix 3741, V-bottom, 1mL Screw Top Storage Tubes (Plasma plate)	Thermo Fisher Scientific 3741

## Off-site Plasma Metabolite Extraction application labware options

The following table lists the approved labware options for each deck location. Some deck locations are limited to one labware type, while other locations can accommodate several labware types.

Deck location	Labware options	Manufacturer part number
1, 5, 8	96 Costar 3961 PP 2ml assay block	Corning Costar 3961
1, 5, 8	96 Agilent A696001000 Captiva collection plate	Agilent A696001000
5, 8	96 Agilent 203426-100 PP, 1 mL Rnd Btm	Agilent 203426-100
5	96 EK 2460 PP Rnd Well U Btm	E&K Scientific EK- 2460
5	96 Greiner 655101 PS Clr Rnd Well Flat Btm	Greiner 655101
6	96 well Captiva EMR-Lipid Filter plate (Captiva EMR - Lipid plate)	Agilent 5190-1001
7, 9	96 V11 LT Tip Box (250 μL disposable pipette tips)	Agilent 19477.002
8	96 Thermo Matrix 3741, V-bottom, 1mL Screw Top Storage Tubes (Plasma plate)	Thermo Fisher Scientific 3741

# Reconstitution application labware options

Deck location	Labware options	Manufacturer part number
1, 7, 9	Tip boxes of 250 µL tips	Agilent 19477.002
	Note: At a minimum, 2 tip boxes are required. If you select the optional addition of a class-specific standard, you need 3 tip boxes.	
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

 $\it Note$ : The deck location 5 labware is required only If you select the optional addition of a class-specific standard.

# Reagent Transfer utility labware options

Deck location	Labware options	Manufacturer part number
5	Tip box of 250 μL tips	Agilent 19477.002
7, 8	96 Costar 3961, PP 2ml assay block	Corning Costar 3961
7, 8	96 EK 2460 PP Rnd Well U Btm	E&K Scientific EK- 2460
7, 8	96 Greiner 655101 PS Clr Rnd Well Flat Btm	Greiner 655101
7, 8	96 Agilent 203426-100 PP, 1 mL Rnd Btm	Agilent 203426-100
7, 8	96 Agilent A696001000 Captiva collection plate	Agilent A696001000
7,8	Reservoir, Seahorse 201254-100, PP, no walls, pyramid bottom	Agilent 201254-100
7, 8	96 Thermo Fisher Scientific 3741 Tube, ScrewTop, 1.0 mL 2D	Thermo Fisher Scientific 3741
7, 8	96 V11 Manual Fill Reservoir	Agilent G5498B#049

## Tip Transfer utility labware

The Tip Transfer utility requires Agilent 96 250-µL pipette tips (Agilent Technologies 19477.002):

- Full tip box at deck location 5
- Empty tip box at deck location 8

## About the installed files

### About this topic

This topic is a reference for personnel who have been trained in the relationships of the various VWorks files.

### Folder structure

The software installer for the Bravo Metabolomics Workbench installs files in the following folder:

...\VWorks Workspace\Metabolomics folder

The Metabolomics folder includes the following folders of files that the VWorks software uses to run the protocols:

• **Device File**. Contains the VWorks device file (.dev) that you use to establish communication with your Bravo Platform.

The device file is associated with a Bravo profile that contains the accessory configuration and teachpoints for your specific Bravo instrument.

- **Form File**. Contains a VWorks form file (.VWForm) for each application and utility. The form provides the user interface for the application or utility.
- Protocol File. Contains a VWorks protocol file (.pro) for each application and utility.

For more details on these file types, see the VWorks Automation Control User Guide.

The Metabolomics folder also contains the following folders, which store image files that are used in the workbench interface:

- Graphics File
- Launch Pad

# Reporting problems

If you find a problem with the Bravo Metabolomics Sample Prep Platform, contact Agilent Automation Solutions Technical Support. See "Contacting Agilent Technologies" on page 2.

To report problems with	Have the following information ready		
Hardware	<ul> <li>Instrument serial number from the Bravo serial number label</li> </ul>		
	Short description of the problem		
Software	<ul> <li>Instrument serial number from the Bravo serial number label</li> </ul>		
	Short description of the problem		
	<ul> <li>Relevant software version number (for example, automation control software, diagnostics software, and firmware)</li> </ul>		
	<ul> <li>Error message text (or screen capture of the error message dialog box)</li> </ul>		
	<ul> <li>Relevant files, such as log files</li> </ul>		