Bravo Metabolomics Workbench

On-site Plasma Metabolite Extraction

Application Guide

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

This guide contains the following topics:

About this guide

Overview

This guide describes the On-site Plasma Metabolite Extraction application for 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 have been trained on how to operate 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 software.

Contacting Agilent Technologies

Web: https://www.agilent.com

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

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App description

The On-site Plasma Metabolite Extraction application performs automated sample preparation on up to 96 plasma samples collected on site in a single protocol run. The protocol performs sample quenching and lipid removal to extract the metabolites, and then normalizes the eluate volume in the final plate.

During the protocol, the Bravo Platform does the following:

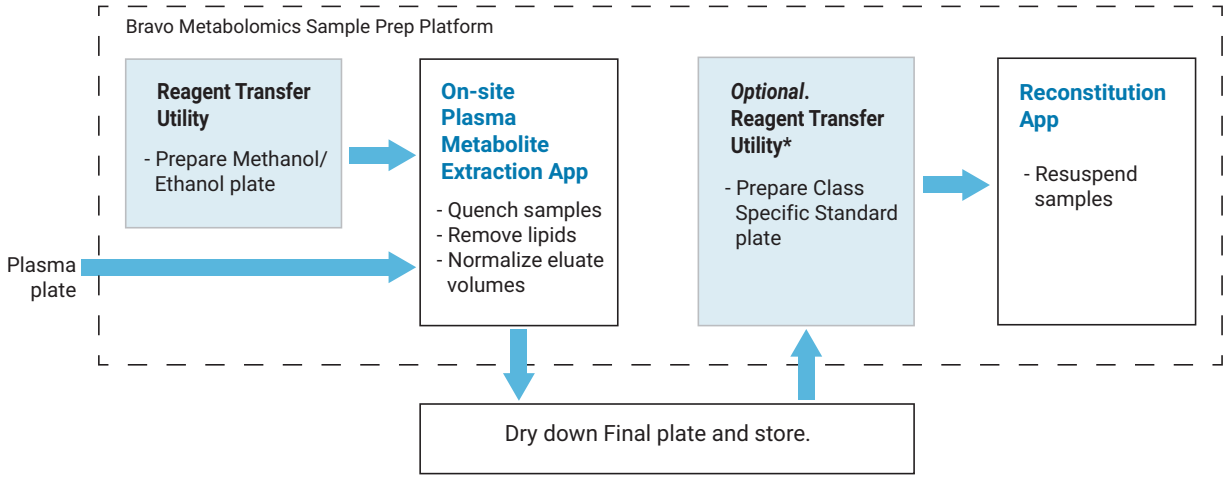
- **Sample quenching**. Transfers plasma from tubes in a 96-well format (Plasma plate) to a prepared Methanol/Ethanol plate. You can use the Reagent Transfer utility to prepare the labware and to add an internal standard, if applicable, to the Methanol/Ethanol plate.

  The protocol can automatically shake and mix the plate on the Bravo deck, or optionally, the protocol will pause and allow you to vortex the plate off the Bravo deck.
- **Lipid removal**. Uses a Captiva EMR-Lipid plate to filter the sample into a Collection plate.
- **Eluate transfer to a Final plate**. Transfers a set volume of eluate from the wells in the Collection plate to a Final plate.

Next steps:

You can dry-down the Final plate in a centrifugal evaporator, such as a Speedvac, for storage. Later, you use the Reconstitution application to resuspend the sample.

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



**Assumes that the user manually pours the methanol and water for the Reconstitution app.*

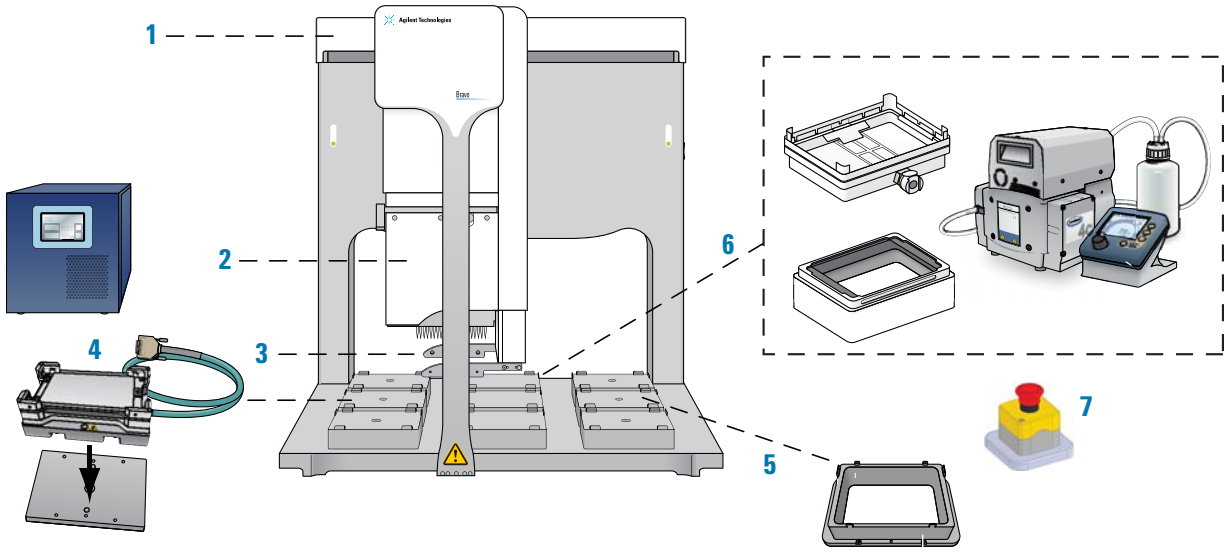
Before you start

This topic lists the required hardware, labware, and reagents for running the protocol.

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 • 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)

Additional equipment

Centrifugal evaporator, such as a SpeedVac, to dry-down the Final plate

If you choose to vortex the samples off the Bravo deck, the vortex equipment is required.

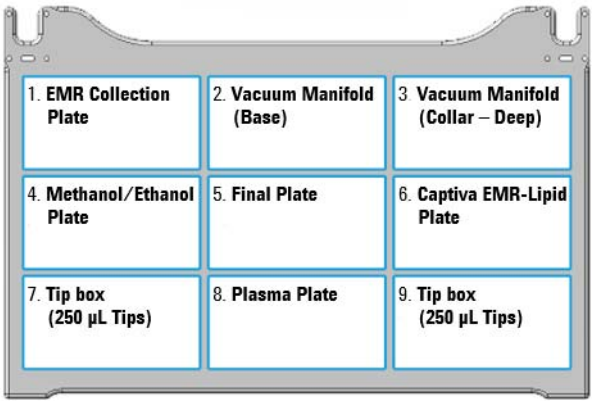
Labware requirements

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.

The following figure shows the nine Bravo deck locations for labware.

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



The following table lists the labware options for each of the nine Bravo deck locations.

Table Labware options by Bravo deck location

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 ScrewTop, Storage Tubes (Plasma plate)	Thermo Fisher Scientific 3741

IMPORTANT The protocol supports full plates or partial plates. Partial plates must be arranged in full, contiguous columns.

Samples and reagents

- Plasma that contains the metabolites to be extracted
- Internal standard

  If required, use the Reagent Transfer utility to premix an internal standard with the Methanol/Ethanol plate before the run.
- Methanol/Ethanol (1:1)

Preparing the sample and reagent labware

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

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

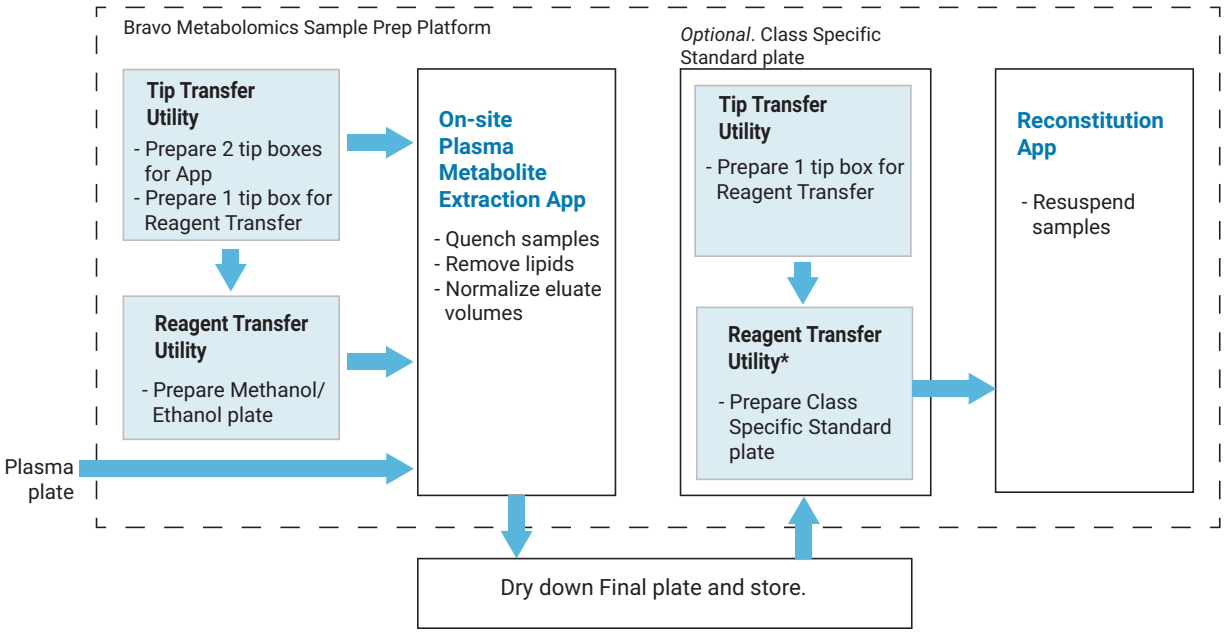
- Fluids are arranged in full columns that are contiguous.
- Plasma columns start with column 1 of the tube rack.
- Methanol/Ethanol columns correspond to the number of Plasma columns you are running.

  Note: The reagent in the Methanol/Ethanol plate may start at any column number.
- Two tip boxes containing sufficient pipette tips for your run:
  - Tips for plasma addition and lipid removal
  - Tips for eluate transfer

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

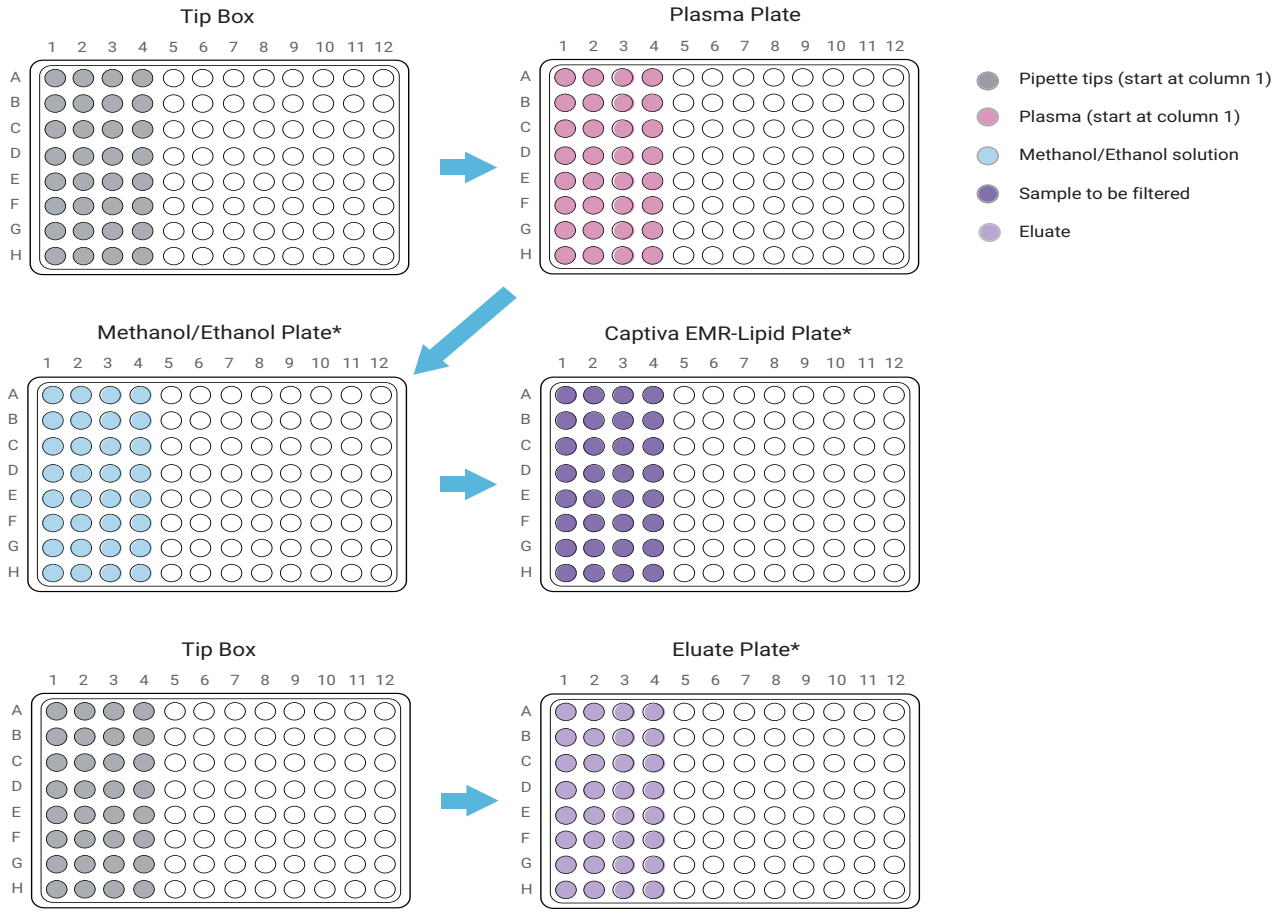
Use the Reagent Transfer utility to transfer the fluids from one labware to another. For partial plate runs, use the Tip Transfer utility to arrange the pipette tips in the tip boxes, as the following figures show.

Figure Example of the workflow for a partial-plate run



See the following figure for an example of the labware layout for a partial-plate run.

Figure Example of labware layout for a run with four columns of plasma tubes



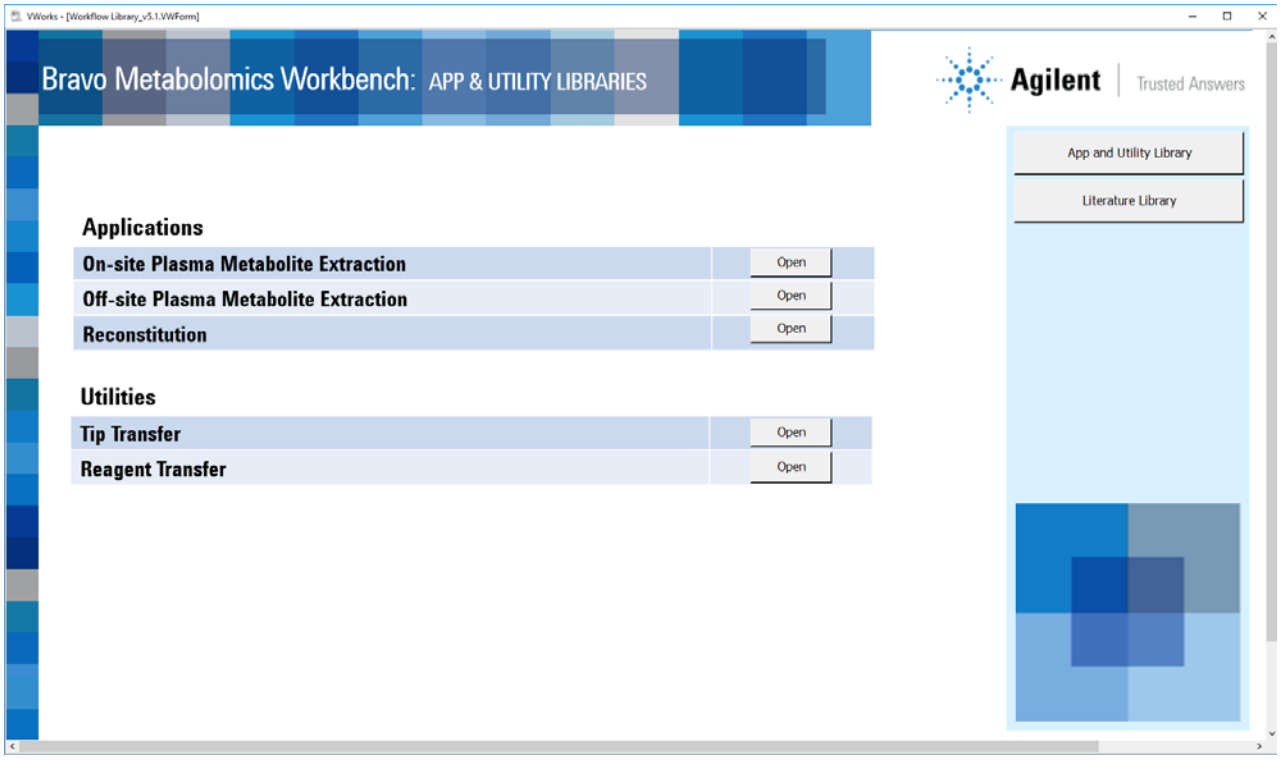
*You can specify the starting column of wells in the Methanol/Ethanol plate, Captiva plate, and Eluate plate.

Setting up the protocol

Opening the application

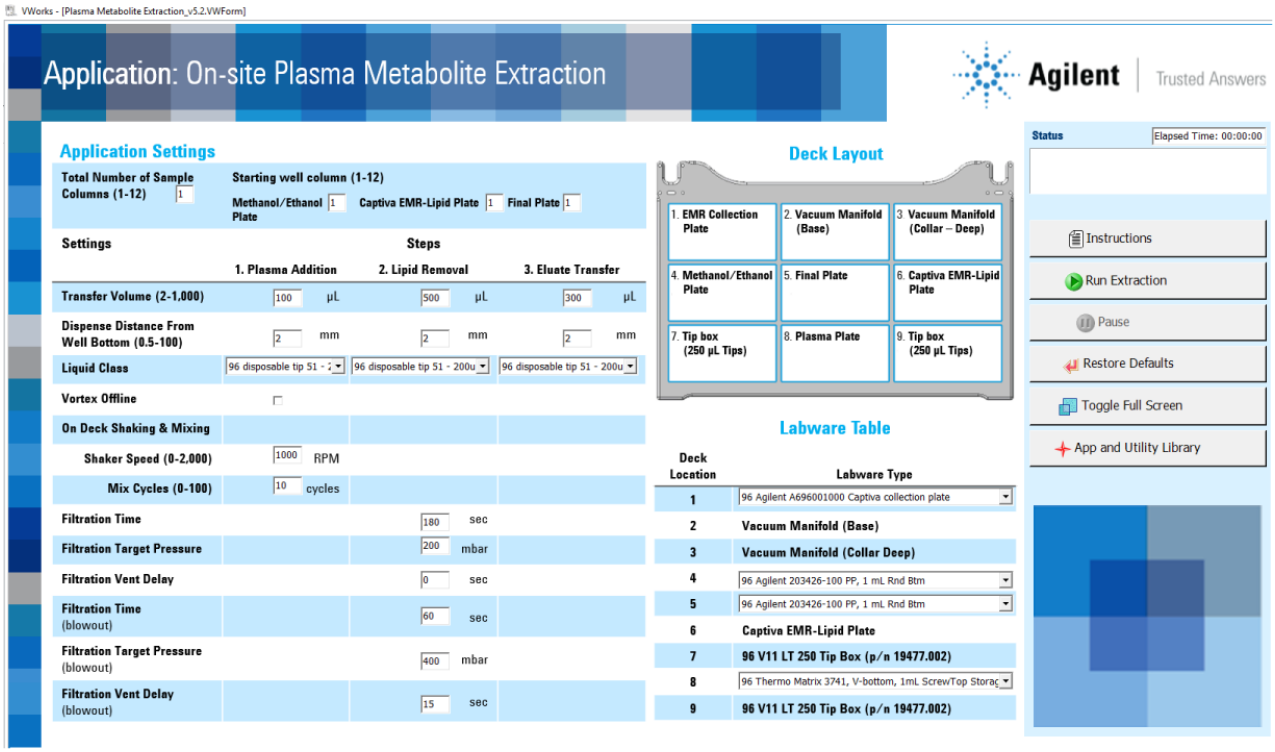
To open the application:

In the **App & Utility Libraries page** of the Bravo Metabolomics Workbench, locate **On-site Plasma Metabolite Extraction**, and then click **Open**.



Specifying the settings

Before starting the protocol, make sure the appropriate selections and values are specified in the On-site Plasma Metabolite Extraction form.



To specify the settings:

1 *Optional*. Click Restore Defaults to set the form settings to their defaults.

2 Under **Application Settings**, specify the following:

Setting	Description
Total Number of Sample Columns (1–12)	The number of columns of plasma samples in the tube rack at deck location 8, for example, type 12 for a full rack of samples. **IMPORTANT** Ensure that the tubes are in full columns that are contiguous, starting with column 1. Default: 1
Starting well column (1–12), Default: 1	
Methanol/Ethanol Plate	The starting column of destination wells in the plate at deck location 4. For a full-plate run, this is column 1. The protocol transfers the sample from the specified number of columns in the Plasma plate to columns in the Methanol/ Ethanol plate, starting at this column number.

Setting	Description
Captiva EMR-Lipid Plate	The starting column of the destination wells in the Captiva plate on the Vacuum Filtration Station at deck location 2. For a full-plate run, this is column 1. The protocol transfers the sample from the Methanol/Ethanol plate to the Captiva plate, starting at this column number.
Final Plate	The starting column of wells in the plate at deck location 5. For a full-plate run, this is column 1. The protocol transfers a set volume of eluate from the EMR Collection plate to the Final plate, starting at this column number.

3 Specify the **Settings** for the following **Steps**:

- Plasma Addition (see "**Plasma Addition**" on page 11)
- Lipid Removal (see "**Lipid Removal**" on page 12)
- Eluate Transfer (see "**Eluate Transfer**" on page 13)

Plasma Addition

Step	Description
Transfer Volume	The volume of liquid per pipette tip to transfer from the Plasma plate (deck location 8) to the Methanol/Ethanol plate (deck location 4). *Note:* 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. Default: 100 (µL) Range: 2–1000 (µL)
Dispense Distance From Well Bottom	The dispense distance between the end of the pipette tips and the well bottoms during the liquid transfer. 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. Default: 2 (mm) Range: 0.5–100 (mm)
Liquid Class	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: • 96 disposable tip 1 -2 µL • 96 disposable tip 2 - 50 µL • 96 disposable tip 51 - 200 µL
Vortex Offline	The option to use off-deck equipment to vortex the sample. To use this option, select the check box. The software ignores the settings for On-Deck Shaking (with Pipette Mixing). During the run, a message will prompt you to move the plate from the Bravo deck to the off-deck vortex mixer.

Step	Description
On-Deck Shaking & Mixing	
Shaker Speed	The shake speed, in revolutions per minute (RPM). Default: 1000 (RPM) Range: 0–2000 RPM)
Mix Cycles	The number of aspirate-and-dispense cycles used to mix the contents of the Methanol/Ethanol plate on the Orbital Shaking Station (deck location 4). Default: 10 Range: 0–100 *Note:* A value of 0 will result in no mix cycles.

Lipid Removal

Step	Description
Transfer Volume	The volume of liquid per pipette tip to transfer from the Methanol/Ethanol Plate (deck location 4) to the Captiva EMR-Lipid plate (deck location 2). Default: 500 (µL) Range: 2–1000 (µL)
Dispense Distance From Well Bottom	The dispense distance between the end of the pipette tips and the well bottoms during the liquid transfer. Default: 2 (mm) Range: 0.5–100 (mm)
Liquid Class	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: • 96 disposable tip 1 -2 µL • 96 disposable tip 2 - 50 µL • 96 disposable tip 51 - 200 µL
Filtration Time	The length of time, in seconds, to leave the vacuum on. At the end of the period, the vacuum will turn off. Default: 180 (s) Range: 0–86400 (s) (24-hour maximum)
Filtration Target Pressure	The difference between the pressure of the outside atmosphere above the filter and the pressure in the Vacuum Filtration Station manifold, including the enclosure beneath the filter. For example, if you set the Target pressure to 600 mbar and the ambient pressure displayed on the VARIO pump is 1000 mbar, the vacuum will remain on until the reading on the VARIO pump reaches 400 mbar. Default: 200 (mbar) Range: 0–10000 (mbar)

Step	Description
Filtration Vent Delay	The length of time, in seconds, to wait for the air pressure under the filter to equalize with the ambient air pressure. Default: 0 (s) Range: 0–86400 (s) (24-hour maximum)
Filtration Blowout	A second filtration task to ensure that all the residual fluid has passed through the Captiva plate (blowout) on the Vacuum Filtration Station.
Filtration Time (blowout)	The length of time to leave the vacuum on. Default: 60 (s) Range: 0–86400 (s) (24-hour maximum)
Filtration Target Pressure (blowout)	The pressure (mbar) to use to filter any remaining residual fluid through the Captiva plate. Typically, this would be a higher value that used for the primary filtration task. Default: 400 (mbar) Range: 0–10000 (mbar)
Filtration Vent Delay (blowout)	The length of time (seconds) to wait for the air pressure under the filter to equalize before the Bravo gripper attempts to disassembly the Vacuum Filtration Station. Default: 15 (s) Range: 0–86400 (s) (24-hour maximum)

Eluate Transfer

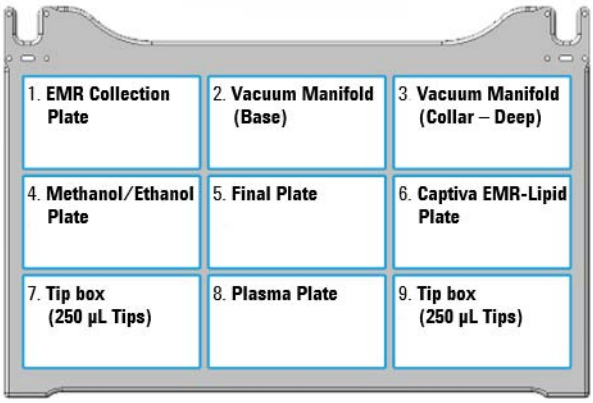
Step	Description
Transfer Volume	The volume of liquid per pipette tip to transfer from the EMR Collection plate (deck location 1) to the Final plate (deck location 5). Default: 300 (µL) Range: 2–1000 (µL)
Dispense Distance From Well Bottom	The dispense distance between the end of the pipette tips and the well bottoms during the liquid transfer. Default: 2 (mm) Range: 0.5–100 (mm)
Liquid Class	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: • 96 disposable tip 1 -2 µL • 96 disposable tip 2 - 50 µL • 96 disposable tip 51 - 200 µL

Specifying the labware

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

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 1, 4, 5, and 8.

Deck Location	Labware Type
1	96 Agilent A696001000 Captiva collection plate
2	Vacuum Manifold (Base)
3	Vacuum Manifold (Collar Deep)
4	96 Agilent 203426-100 PP, 1 mL Rnd Btm
5	96 Agilent 203426-100 PP, 1 mL Rnd Btm
6	Captiva EMR-Lipid Plate
7	96 V11 LT 250 Tip Box (p/n 19477.002)
8	96 Thermo Matrix 3741, V-bottom, 1mL ScrewTop Storag
9	96 V11 LT 250 Tip Box (p/n 19477.002)

Running the protocol

Before you start

- Prepare the samples and reagents. See "Preparing the sample and reagent labware" on page 7.
- Ensure that you have two boxes of fresh pipette tips containing the required tips for your run:
  - **Full-plate run**. Use two full tip boxes.
  - **Partial-plate run**. Two tip boxes with the corresponding number of pipette tips arranged in contiguous columns starting at column 1.

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, mix cycles, and filtration time.

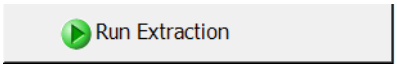
Starting the protocol run

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 

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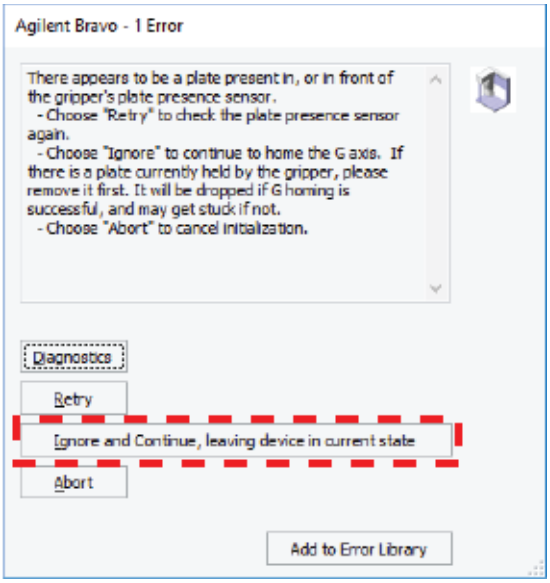
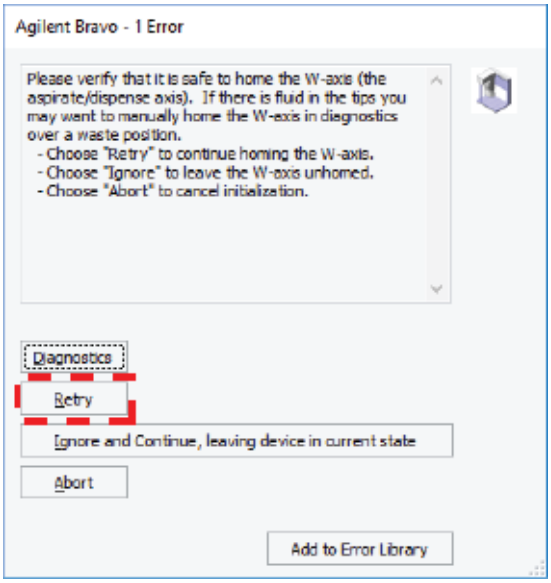
- If this is the first time the application has been run after powering up the Bravo Platform, the device initialization process begins. Proceed to step 4.
- If the platform is already initialized, skip to step 6.

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.



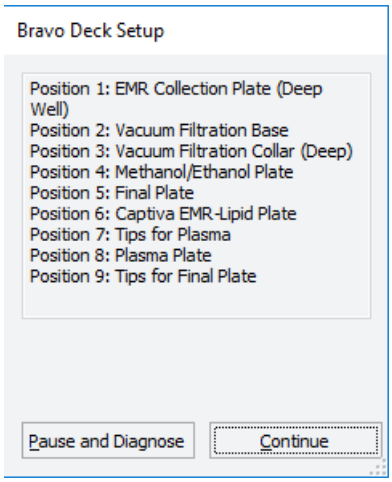
5 If the **Please verify that it is safe to home the W-axis** message appears, click **Retry** to continue homing the pipetting axis (*w*-axis).



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.

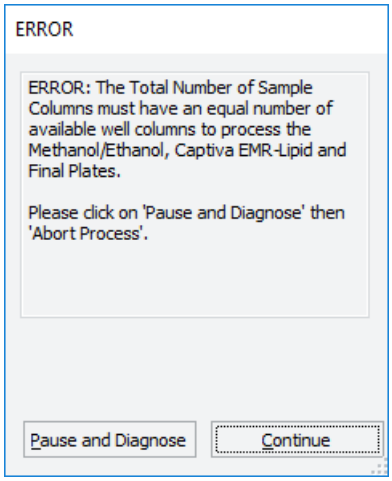
- If it is correct, click **Continue**.
- If it is not correct, click **Pause and Diagnose**, and then click **Abort process** in the Scheduler Paused dialog box. Resolve the problem, and then restart the run.



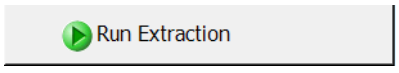
7 If an Error message appears, the software detected a conflict in the protocol setup.

a Follow the on-screen instructions to click **Pause and Diagnose** and then click **Abort process** in the Scheduler Paused dialog box.

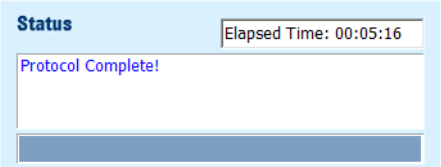
b Resolve the conflict described in the error message, for example:



- 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
Vacuum Filtration Station Assembly	1, 2	Moves the EMR Collection plate to the manifold base at deck location 2.
	3, 2	Moves the Deep Well Collar to the manifold base at deck location 2.
	6, 2	Moves the Captiva plate to the Vacuum Filtration Station at deck location 2.
Tip Pick Up	7	Presses on the columns of pipette tips from the tip box. The number of full columns corresponds to the specified number of columns in the Plasma plate.
Plasma Transfer	8, 4	Transfers the sample from the Plasma plate into the specified columns of wells in the Methanol/Ethanol plate on the Shaking Station. *Note:* The transfer cycle may repeat depending on the volume to be transferred.
Shaking and Mixing	4	Depending on the option you selected:
		• **Vortex Offline option**. A message displays and tells you to remove the plate for offline vortexing.
		• **On Deck Shaking & Mixing option**. Shakes the plate at the specified speed and performs the selected number of mix cycles.
Sample Transfer	4, 2	Transfers the sample from the Methanol/Ethanol plate into the specified wells of the Captiva plate. *Note:* The transfer cycle may repeat depending on the volume to be transferred.
Filtering	2	Filters the sample through the Captiva plate into the EMR Collection plate at the Vacuum Filtration Station.
Tip Removal	7	Ejects the used pipette tips into the tip box on deck location 7.
Vacuum Filtration Station Disassembly	2, 6	Moves the Captiva plate to deck location 6.
	2, 3	Moves the Deep Well Collar to deck location 3.
	2, 1	Moves the EMR Collection plate to deck location 1.
Tip Pick Up	9	Presses on the columns of pipette tips from the tip box. The number of full columns corresponds to the specified number of columns in the Final plate.

Protocol step	Head moves to deck location...	Action
Sample Transfer	1, 5	Transfers a specified volume of sample from the EMR Collection plate into the specified wells of the Final plate. *Note:* The transfer cycle may repeat depending on the volume to be transferred.
Tip Removal	9	Ejects the used pipette tips into the tip box at deck location 9.