

# Bravo Metabolomics Workbench

## Reagent Transfer

### User Guide

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



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## About this guide

### Overview

This guide describes the Reagent Transfer utility 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 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 software.

### Contacting Agilent Technologies

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

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

Documentation feedback: [documentation.automation@agilent.com](mailto:documentation.automation@agilent.com)

## Utility description

The Reagent Transfer utility transfers samples or reagents from a Source Plate into a Destination Plate. This utility is useful for sample manipulation and reagent preparation operations.

## Before you start

### Hardware requirements

The Reagent Transfer utility requires the Bravo Metabolomics Sample Prep Platform. For a description of the components, see the [Bravo Metabolomics Sample Prep Platform Getting Started Guide](#).

### Labware requirements

#### CAUTION

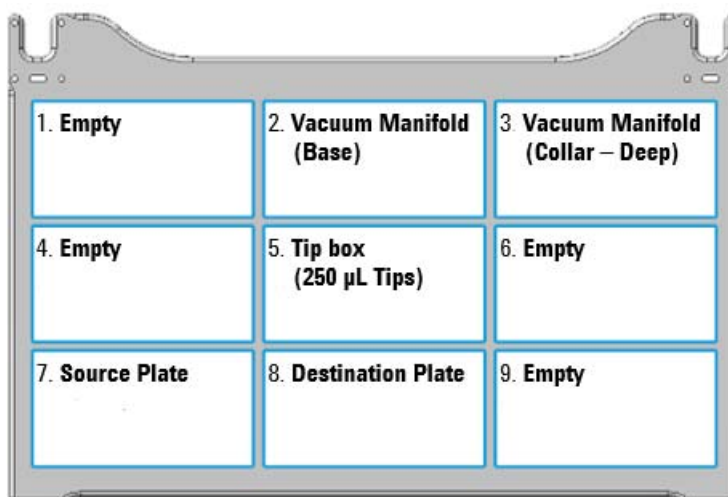
Using a labware type at a deck location other than an approved labware option can cause a collision resulting in equipment damage. Ensure that you use only an approved labware option for each deck location.

The Reagent Transfer utility requires the following labware:

- Tip box of Agilent 96 250- $\mu$ L pipette tips (part number 19477.002) at deck location 5
- Source and destination plates (deck locations 7 and 8)

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

**Figure** Reagent Transfer utility labware locations on the Bravo deck (top view)



The following table lists the approved labware options for each deck location.

**Table** Labware options by deck location

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

## Preparing the labware

### IMPORTANT

The protocol supports only full, contiguous columns in the labware.

Ensure that the labware is prepared as follows:

- Fluids arranged in full columns that are contiguous
- Tips arranged in columns in the tip box starting with column 1

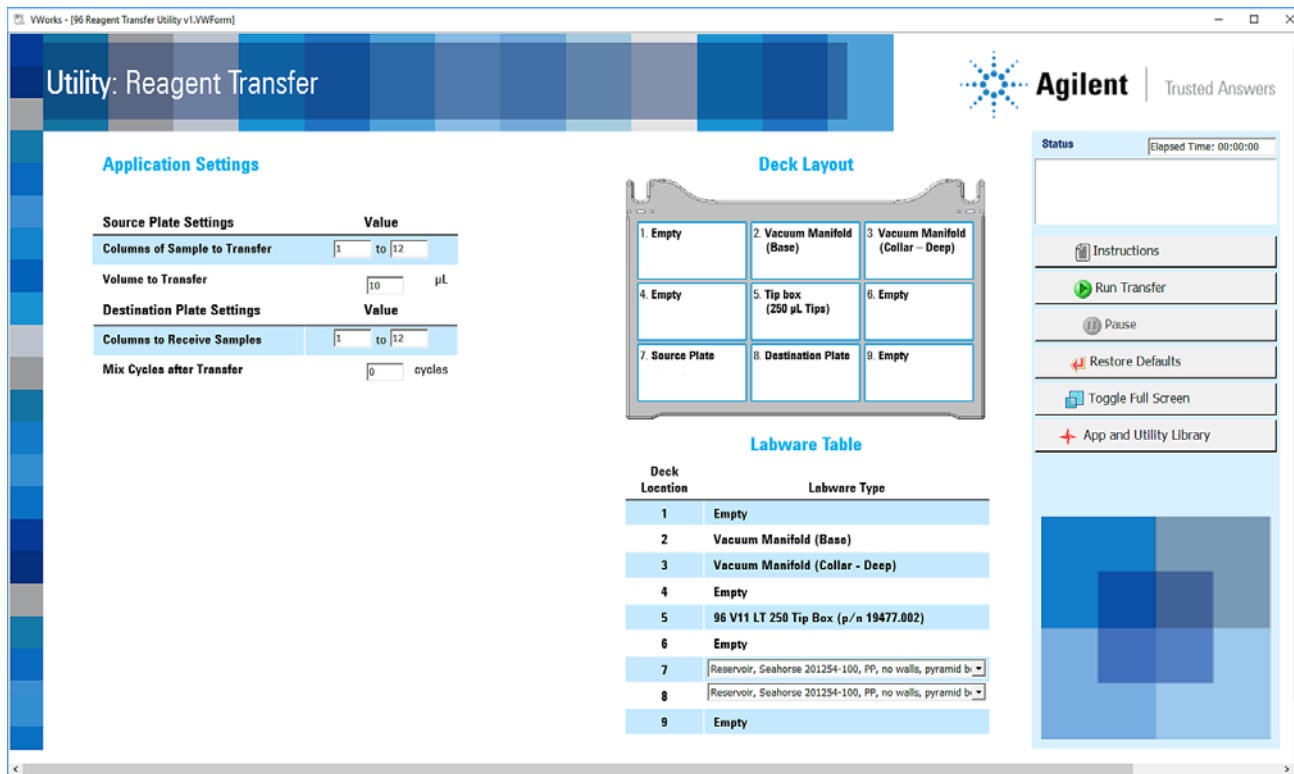
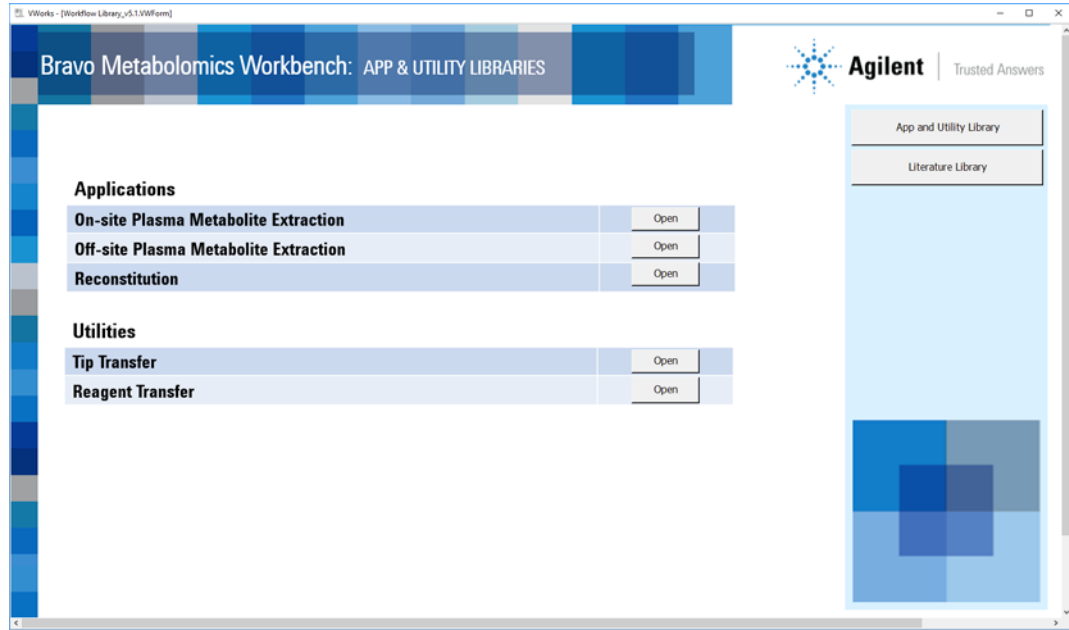
If you are using a partially filled tip box, you can use the Tip Transfer utility in the workbench to arrange the pipette tips.

The number of columns in the tip box should correspond to the number of columns in the Source Plate and Destination Plate.

## Accessing the utility

**To open the utility form:**

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



## Setting up the protocol

### Specifying the settings

#### To specify the settings:

1 Under **Application Settings**, specify the following **Source Plate Settings**:

Setting	Description
Columns of Sample to Transfer (1–12)	<p>The range of wells (full columns) in the Source Plate (deck location 7) to be used for the reagent transfer.</p> <p>Ensure that the sample is in full columns that are contiguous.</p> <ul style="list-style-type: none"><li>In the left box, type the number of the first column of filled wells.</li></ul> <div data-bbox="683 699 896 764" style="border: 1px solid #ccc; padding: 2px; display: inline-block;"><input style="width: 30px; height: 20px; border: 1px solid #ccc;" type="text" value="1"/> to <input style="width: 30px; height: 20px; border: 1px solid #ccc;" type="text" value="12"/></div> <ul style="list-style-type: none"><li>In the right box, type the number of the last column of filled wells.</li></ul> <p>Default: 1 to 12</p>
Volume to Transfer	<p>The volume of liquid per pipette tip to transfer from the source to the Destination Plate.</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>Default: 100 (µL)</p> <p>Range: 0–1200 (µL)</p>

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

Setting	Description
Columns to Receive Samples (1–12)	<p>The range of full columns in the Destination Plate (deck location 8) to which the samples will be transferred.</p> <p>This range may differ from the range specified for the <b>Columns of Samples to Transfer</b> value, but it must contain the same number of full columns. If not, and the software displays an error message, and the protocol automatically aborts.</p> <ul style="list-style-type: none"><li>In the left box, type the number of the first column of destination wells.</li></ul> <div data-bbox="764 1612 977 1677" style="border: 1px solid #ccc; padding: 2px; display: inline-block;"><input style="width: 30px; height: 20px; border: 1px solid #ccc;" type="text" value="1"/> to <input style="width: 30px; height: 20px; border: 1px solid #ccc;" type="text" value="12"/></div> <ul style="list-style-type: none"><li>In the right box, type the number of the last column of destination wells.</li></ul> <p>Default: 1 to 12</p>

Setting	Description
Mix Cycles after Transfer	<p>The number of aspirate-and-dispense cycles used to mix the contents of the Destination Plate (deck location 8) after the transfer has been completed.</p> <p>The volume used for the mixing process is automatically calculated as follows:</p> <ul style="list-style-type: none"> <li>If transfer volume &gt; 250 (tip capacity), then mix volume is 245 <math>\mu</math>L</li> <li>If transfer volume is 50–245 <math>\mu</math>L, then mix volume = 75% of transfer volume</li> <li>If transfer volume is &lt;50 <math>\mu</math>L, then mix volume = 50% of transfer volume</li> </ul> <p>Default: 0 Range: 0–20</p>

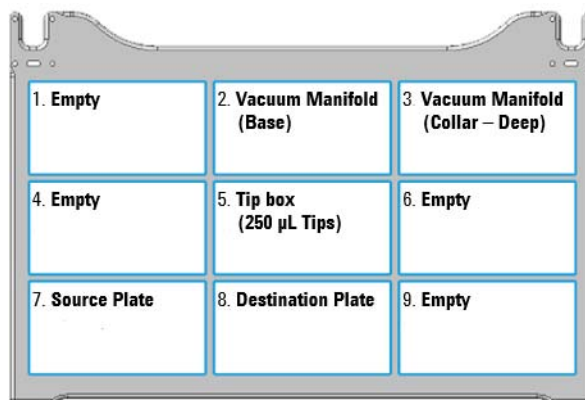
## Specifying the labware

### 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 specifying 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 7 and 8.

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

## Running the protocol

### 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.

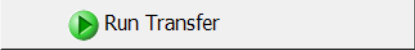
### Starting the protocol run

#### *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.

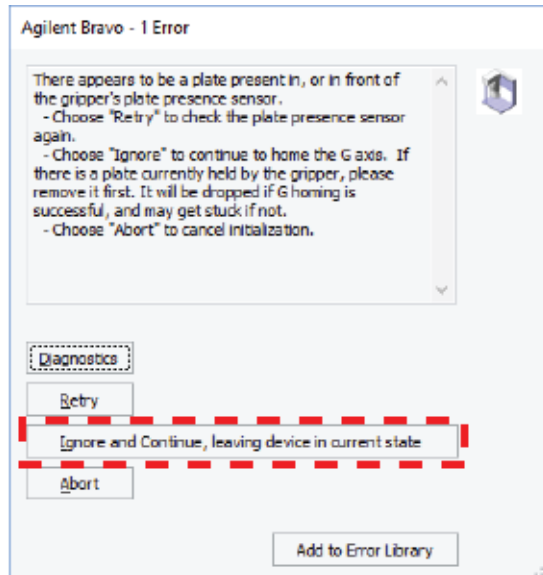
#### CAUTION

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.

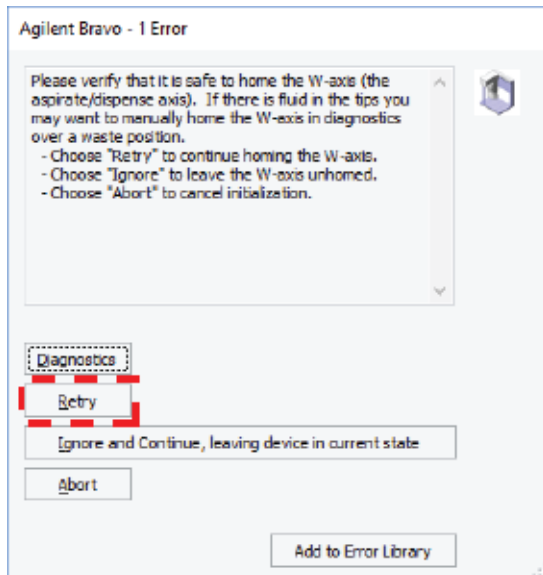
- 3 Click  to start the protocol run.
  - If this is the first time the utility has been run after powering up the Bravo Platform, the device initialization process begins. Proceed to step 4.
  - If the platform is already initialized, skip to step 6.



- 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.

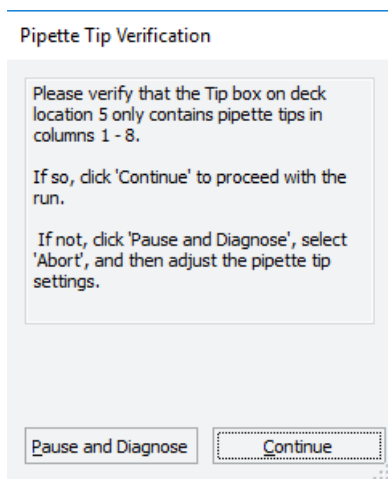


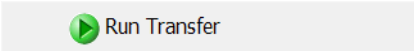
- 5 If the **Please verify that it is safe to home the W-axis** message appears, click **Retry** to continue homing the pipetting axis (*w*-axis).



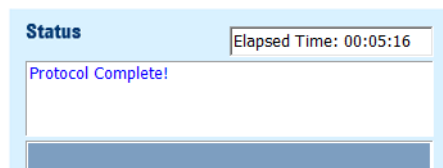
When the initialization process is finished, the orange lights on the Bravo Platform light panel flicker briefly and then begin to flash.

- 6 When the **Pipette Tip Verification** dialog box appears, follow the on-screen instructions to verify that the pipette tips are in the specified columns of the tip box.



- 7 If an Error message appears, the software detected a conflict in the protocol setup.
- Follow the on-screen instructions to click **Pause and Diagnose** and then click **Abort process** in the Scheduler Paused dialog box.
  - Resolve the conflict described in the error message, for example, unequal number of columns selected for source and destination plates.
  - 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 automation movements of the Bravo Platform during the Reagent Transfer run.

Protocol step	Head moves to deck location...	Action
Tip Pick Up for Sample Transfer	5	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.
Aspirate from Source Plate	7	Aspirates the specified volume to transfer from the specified columns in the Source Plate.
Tip Touch	7	Performs a tip touch on the left and right sides of the wells in the Source Plate.
Dispense to Destination Plate	8	Dispenses the sample volume into the specified columns of wells in the Destination Plate.
Repeat Transfer for Volume >250 $\mu$ L	7, 8	Repeats the Aspirate and Dispense steps until the full volume specified in the Volume to Transfer setting has been transferred to the Destination Plate.
Mix	8	Performs the specified number of aspirate-and-dispense cycles in the wells of the Destination Plate to mix the contents.
Tip Touch	8	Performs a tip touch on the left and right sides of the wells in the Destination Plate.
Eject Tips	5	Ejects the used pipette tips into the tip box at deck location 5.