EVOSEP

Step-by-step guide

Fully automated sample loading on Evotip Pure with the AssayMAP Bravo

The fully automated sample loading protocol for the Agilent AssayMAP Bravo robot makes use of a sandwich approach with defined air gaps between the liquid layers (Figure 1). This is then pushed through the Evotip by applying positive air pressure using the 96 head with a detachable sealing mat. The protocol allows the user to prepare 96 Evotips in ~4 minutes. and requires an Agilent AssayMAP Bravo loading kit (Evosep, EV1145). Additionally, a solvent plate (Thermo Scientific, 2412811) and a sample plate (Eppendorf, 0030129512) is needed.

DECK LAYOUT

1. The protocol requires an Agilent AssayMAP Bravo loading kit (Evosep, EV1145) that consists of two ‘Universal Evotip adapters’, an ‘Evotip 96-head plate’, an ‘Evotip 96-head sealing mat’ and an ‘Evotip tool holder’ together with associated well plates. Additionally, a solvent plate (Thermo Scientific, 2412811) and a sample plate (Eppendorf, 0030129512) is needed.
2. Place one ‘Universal Evotip adapter’ on top of the deep well plate, place a yellow holder with Evotips inside and place it in position 7.
3. Place one ‘Universal Evotip adapter’ on top of the low single well plate, add 80 ml propanol in the plate and place it in position 8.
4. Add 80 ml solvent A (MS-grade water with 0.1% Formic Acid, JT Baker P/N: JT-9834-2 or equivalent) in a low single well plate and place in position 9.
5. Prepare the sample plate, preferably minimum 30 µl per well. 20 µl will be loaded on the Evotip, and place in position 3.
6. Place 250 µl tips (Agilent, 19477-002) in positions 5 and 6.
7. Place the ‘Evotip tool holder’ on top of the Agilent tip loading station in position 2 and place the ‘Evotip 96-head sealing mat’ inside with the ‘Evotip 96-head plate’ on top within the ‘Evotip tool holder’.Make sure to tighten the screws on the side of both the ‘Evotip tool holder’ and on the side of deck position 2.

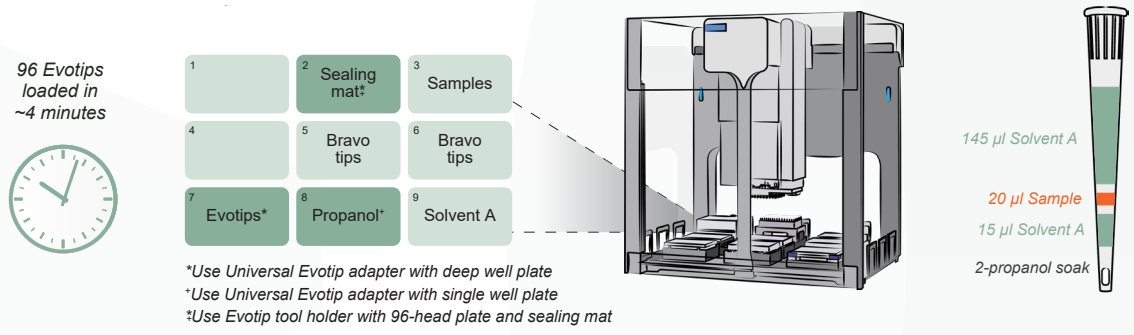

Figure 1: Workflow for automated Evotip loading using the AssayMAP Bravo.

EVOSEP

STEP-BY-STEP GUIDE

8. Download the protocol named ‘AGILENT ASSAYMAP BRAVO – FULL HEAD, AUTOMATED PROTOCOL (ASSAYMAP HEAD)’ from *https://www.evosep.com/automation-agilent-assaym-ap-bravo/*
9. Open VWorks software and import the .vzp protocol file including all labware entries, labware classes, liquid classes, pipette te chniques, device profiles, JSWrapper tasks and error handlers.
10. Click ‘Reinitialize devices’.
11. Perform a ‘Dry run’ and verify (1) that the gripper picks up the Evotips from position 7 to 8 and back, (2) that the AssayMAP head picks up the sealing mat in position 2 and seals on top of the Evotips, and (3) that the sealing mat are detached correctly in position 2.
12. Perform a ‘Wet run’, by adding a minimum of 18 ml of solvent A in positions 3 and 9, and check that the sandwich is created in all the Evotips, during the ‘gripper pick-up’ from position 7 to 8. It is also possible to pause the robot after step 4, to evaluate that the sandwich has been successfully created with visible airgaps between the layers.
13. If step 12 og 13 worked succesfully, you are now ready to run the samples.

ADDITIONAL INFORMATION

I want to run less than 96 samples?
If you want to run less samples than 96, you just need to adjust the number of evotips in the rack and the pipette tips for both solvent A and sample.

My “Dry run” did not work?
If you observe either that the gripper is not picking up the Evotip rack at position 7 or 8 an adjustment of the teach points for the specific position is needed. If the gripper goes further down and thus not “detects” the Evotip rack the teach point needs to be elevated (Go to Diagnostics, Jog/Teach and select the location that needs to be adjusted, slightly adjust the Z-axis either up or down depending on the observed misalignment).
If you observe that the 96 head does not pick up the sealing mat and reports the error “Exceeded destination position on the Z-axis” also an adjustment of the teach point for this location is needed, here you have to slightly decrease the teach point for location 2.

My “Wet run” did not work?
If you observe that a layer is not created between the sample and the upper layer, check in subprocess 13 in the main protocol section in Vworks, that the dispense task parameters under liquid class has the “96_sandwich” assigned. (This class helps to dispense at the side of the tips)
If you observe that no airgap is present between the lower layer and the sample an adjustment of the teach points for the specific position is needed. If the gripper goes further down and thus not “detects” the Evotip rack the teach point needs to be elevated (Go to Diagnostics, Jog/Teach and select the location that needs to be adjusted, slightly adjust the Z-axis either up or down depending on the observed misalignment).

I get ‘Location incompatible with labware’ or ‘Could not find in labware database’ errors?
Re-import the vzp. Protocol and make sure to mport all labware entries, labware classes, liquid classes, pipette techniques, device profiles, JSWrapper tasks and error handlers.