EVOSEP

Step-by-step guide

A complete, automated Opentrons OT-2 loading protocol

As a step towards end-to-end workflows, a simple and automation-friendly protocol for sample loading on Evotips has been developed. It makes use of a sandwich approach with defined air gaps between the liquid layers (Figure 1). This is then pushed through the Evotip with the OT-2 pipette for 100 seconds leaving the Evotip ready for injection on the Evosep One. The protocol has been converted into an easy-to-use HTML form, that generates a complete python script for use in the Opentrons app. It allows the user to load from 8 to 288 Evotips in a single run, requiring an Evotip Opentrons Loading kit (Evosep, EV1144) for each box of Evotips loaded at the time. Additionally, a solvent plate (USA Scientific, 1061-8150) and a sample plate (Eppendorf, 0030129512) is needed.

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1 Prepare solvent plate with 18 ml solvent A and 6 ml 2-propanol for each Evotip box.The first Evotip box uses columns 1 and 12, the second Evotip box uses columns 2 and 11, and the third Evotip box uses columns 3 and 10.

2 Prepare sample plates, preferably minimum 30 µl per well. 20 µl will be loaded on the Evotip.

3 Prepare the remaining OT-2 deck as outlined below (Figure 1) with the Evotips placed in the appropriate adapter (Evosep, EV1144).

4 Go to *https://ot2-evotip-loading.evosep.com/* and select the parameters for the protocol by navigating through the options. Click 'Download'. For the Opentrons app, version 6.3.0 and higher, additional files as indicated are required to be downloaded and imported into the app.

5 Open the Opentrons App and go to the 'Protocols' tab. Import the generated protocol.

6 Open the protocol in the Opentrons app, click 'start setup', select your Opentrons and click 'Proceed to setup'. Calibrate pipettes if needed (Step 1). The protocol will now be analyzed on the robot.

7 Navigate to Labware setup (Step 2). Click 'Apply stored data' and run 'Labware Position Check'.

8 Click 'Start run' and follow the run log for current activity. Once completed, the Evotips can be transferred to the Evotip box, submerged in solvent A and stored at 4 °C until analysis.

Figure 1: Layout of OT-2 deck for automation of Evotip Pure sample loading.

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