

# 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 Evtips 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 Evtip with the OT-2 pipette for 100 seconds leaving the Evtip 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 Evtips in a single run, requiring an Evtip Opentrons Loading kit (Evosep, EV1144) for each box of Evtips loaded at the time. Additionally, a solvent plate (USA Scientific, 1061-8150) and a sample plate (Eppendorf, 0030129512) is needed.

### STEP-BY-STEP GUIDE

- 1 Prepare solvent plate with 18 ml solvent A and 6 ml 2-propanol for each Evtip box. The first Evtip box uses columns 1 and 12, the second Evtip box uses columns 2 and 11, and the third Evtip box uses columns 3 and 10.
- 2 Prepare sample plates, preferably minimum 30  $\mu$ l per well. 20  $\mu$ l will be loaded on the Evtip.
- 3 Prepare the remaining OT-2 deck as outlined below (Figure 1) with the Evtips placed in the appropriate adapter (Evosep, EV1144).
- 4 Go to <https://ot2-evtip-loading.evosep.com/> and select the parameters for the protocol by navigating through the options. Click 'Download'.
- 5 Open the Opentrons App and go to the 'Protocols' tab. Import and run the generated protocol.
- 6 Choose the Opentrons to use for the loading. Calibrate pipettes if needed (Step 1).
- 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 Evtips can be transferred to the Evtip box, submerged in solvent A and stored at 4  $^{\circ}$ C until analysis.

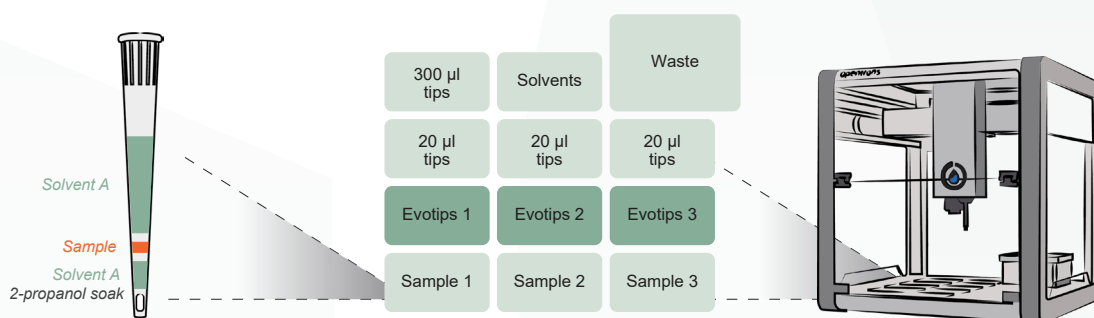


Figure 1: Layout of OT-2 deck for automation of Evtip Pure sample loading.