

SOP #	WL-25-014
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# SOP 014 – Opentrons

15.07.2025

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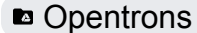
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## 1. Scope

This SOP describes the procedure for using the Opentrons automation hardware and software. The SOP includes details about designing the protocol via Opentrons online user interface (instead of python coding).

## 2. Summary of Methods

Design the protocol in Opentrons user interface or download existing one from the  folder. Import it, connect to the robot via your PC and carry out calibration steps (if needed). Run the protocol.

## 3. Definitions

NA.

## 4. Health and Safety

When working in the multi-laboratory in Skylab, lab coats are always worn. It is not permitted to take anything out of the GMO lab and transport to the multi-lab. Wash your hands thoroughly before exiting the laboratory.

## 5. Personnel Qualifications

All DTU Biobuilders team members that have participated in the relevant safety training.

## 6. Equipment

This should list all the equipment required for the procedure.

- PC,
- Opentrons OT-2 (GEN2),
- 1-channel adapter for 300 uL,
- 8-channel adapter for 20 uL,
- Liquid reservoirs (either 1 or 12-well),
- Any additional module such as the thermocycler.

## 7. Materials

This should list all the consumables to be used in the procedure.

- Relevant SOP's chemicals.

## 8. Procedure

### 8.1 Download and connect the Opentrons software

8.1.1 Go to Opentrons website to download the software for your PC (<https://opentrons.com/ot-app>).

8.1.2 Use the USB from the Opentrons to connect your PC to Opentrons.

8.1.3 Go to the 'Devices tab' within the software and connect to our Opentrols hardware (Figure 1).

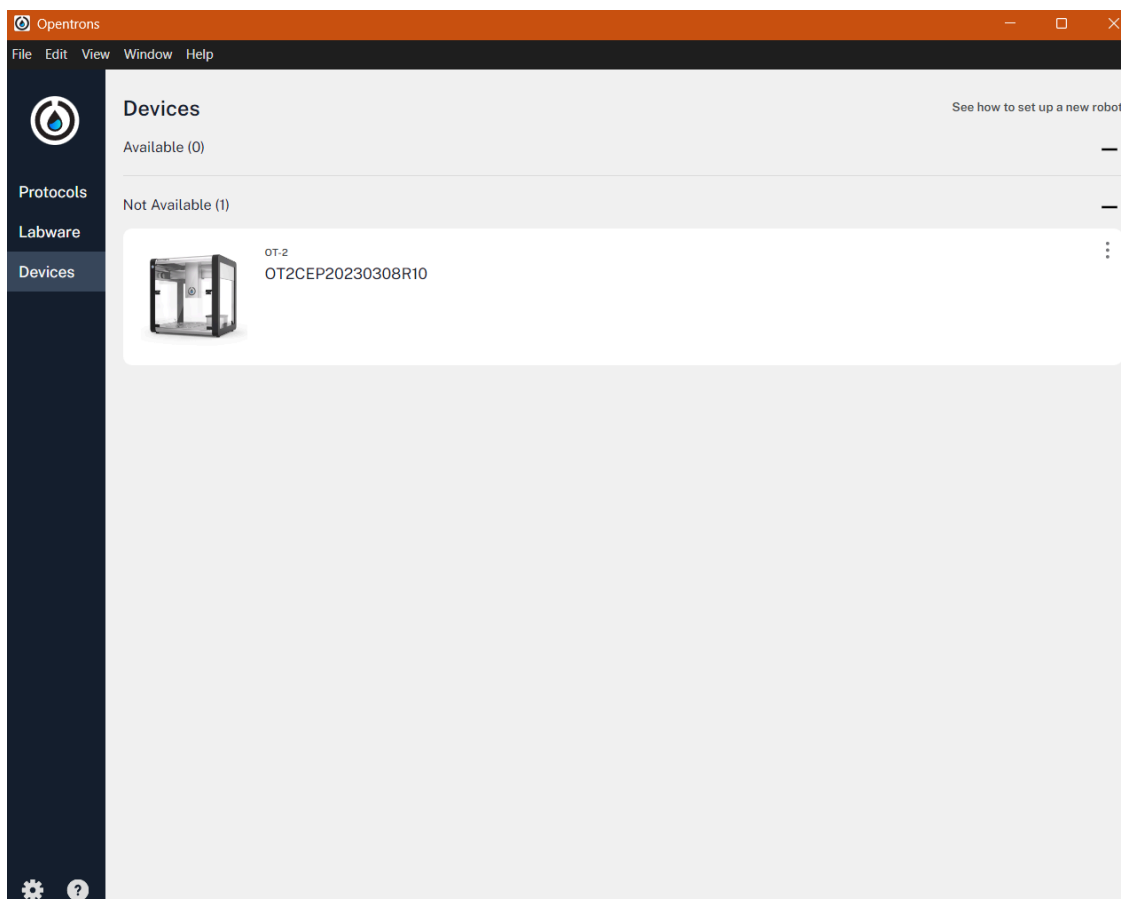


Figure 1. Opentrons software, specifically the devices tab where the Opentrons hardware can be connected to your PC.

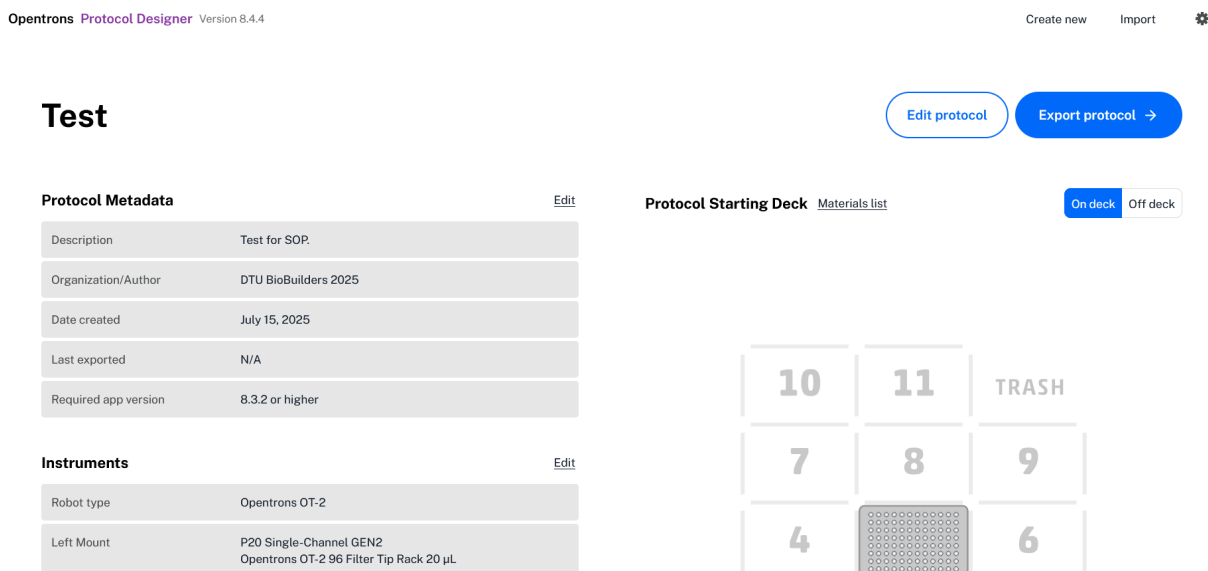
### 8.2 Create the protocol

8.2.1 Use <https://designer.opentrons.com/#/designer> to create a new protocol using an interactive user interface.

- 8.2.1.1 Choose Opentrons OT-2 as the robot.
- 8.2.1.2 Choose 1-channel pipette of GEN2 and 20 uL volume (Filter Tip Rack 20 uL).
- 8.2.1.3 Add 8-channel pipette of GEN2 and 300 uL volume (Filter Tip Rack 200 uL).
- 8.2.1.4 Add relevant modules, such as 'Temperature Module GEN2' etc.
- 8.2.1.5 Name the protocol in a descriptive and informative way. Add any description if needed. Label the Author/Organization as 'DTU BioBuilders 2025'.

8.2.2 Define the hardware and liquids for the protocol.

- 8.2.2.1 Press 'Edit protocol' and click on a slot that will have your required hardware (Figure 2). Define positions of your tip racks, reservoirs (for media etc) and any other modules by clicking on the slot, then 'Add hardware/labware and pick your desired equipment.



The screenshot shows the Opentrons Protocol Designer interface. At the top, it says "Opentrons Protocol Designer Version 8.4.4" and has buttons for "Create new", "Import", and a settings icon. Below this is a "Test" section with "Edit protocol" and "Export protocol" buttons. The main area is divided into two panels: "Protocol Metadata" and "Protocol Starting Deck".

**Protocol Metadata** (with an "Edit" link):

Description	Test for SOP.
Organization/Author	DTU BioBuilders 2025
Date created	July 15, 2025
Last exported	N/A
Required app version	8.3.2 or higher

**Protocol Starting Deck** (with a "Materials list" link and "On deck" / "Off deck" toggle):

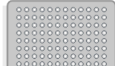
10	11	TRASH
7	8	9
4		6

Figure 2. Opentrons interactive user interface for online design of protocols.

- 8.2.2.1.1 You can add liquid into reservoirs by first adding a reservoir into a slot and then clicking 'Add liquid'. Then click on the reservoir well and choose liquid name and volume. If needed, click 'define liquid' to add more. Click 'Save' and then 'Done' (Figure 3).



Figure 3. Opentrons interactive user interface for online design of protocols, specifically definition of liquid cultures.

8.2.2.1.2 In the top left corner, click 'Protocol steps'. In the bottom left corner, click 'Add Step'. To transfer liquid, click 'Transfer'. A tab will appear on the right side.


8.2.2.1.2.1 You can change the step name to be more descriptive.

8.2.2.1.2.2 Make sure to define the source location on the deck, as well as which well within the reservoir the robot should use. Similarly, define the destination location.

8.2.2.1.2.3 Define the volume to be transferred.

8.2.2.1.2.4 Click 'Continue' and, if needed, edit the 'Aspirate' and 'Dispense' tabs.

8.2.2.1.2.5 Save and continue onto the next steps within your SOP of choice until done.

8.2.2.1.2.6 Click done at the top right corner. Then click 'Export protocol' to save the file. Upload to  Opentrons folder for safekeeping and so that the protocol is available for others.

### 8.3 Import the protocol and calibrate Opentrons before running the new protocol

8.3.1 Within the Opentrons Software, go to the 'Protocols' tab. In the top right corner, press 'Import'. Choose your protocol file and open it.

8.3.2 Click on 'Start setup'. Choose our robot and if available, use available off set data. This way you will not need to re-calibrate the robot. Otherwise, follow the software's guidance to recalibrate the robot.

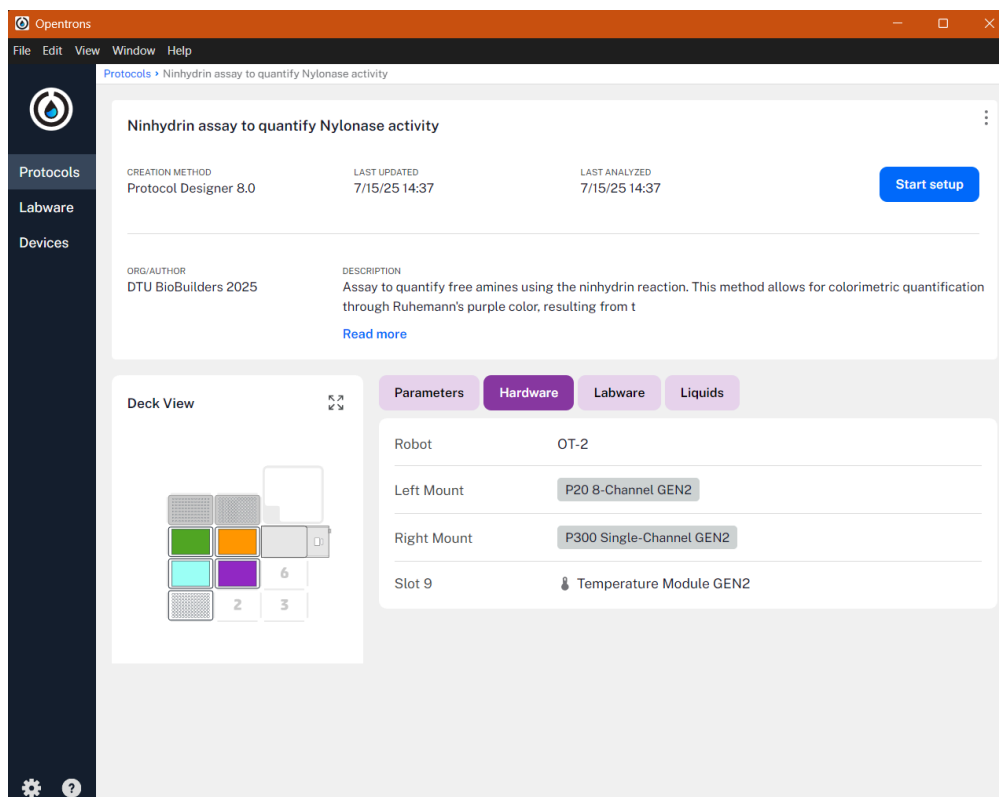


Figure 4. Opentrons software, specifically the protocols tab.

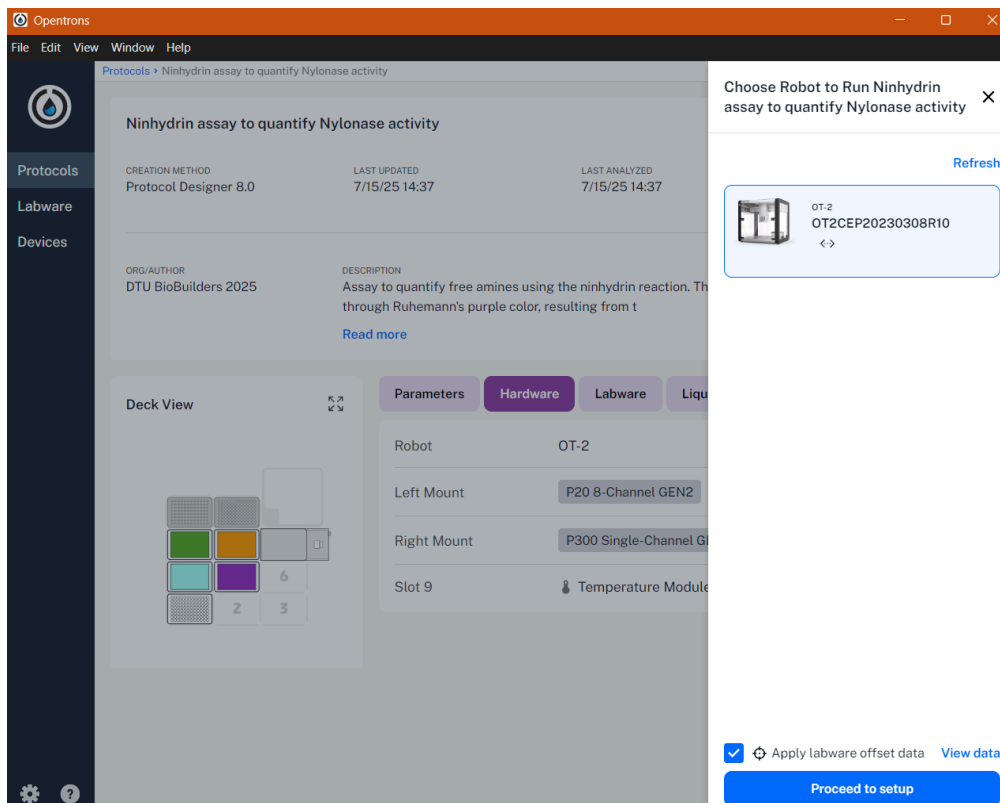


Figure 5. Opentrons software, specifically the protocols tab with the offset data available.

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- 8.3.2.1 **IMPORTANT:** calibrating tip positions is important, **but do not** calibrate the makeshift tip rack lids - use the preset settings.
- 8.3.3 If this is your first time running the protocol, test check it with food dyes instead of real chemicals. Make sure to take notes in case future fixes are required. Use Opentrons online user interface to fix the protocol as required (i.e. step 8.2).

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## 9. Data and Records Management

Save all protocols in the **Opentrons** folder.

## 10. References

NA.

## 11. Appendix

NA.