Apr 15, 2020

# Opentrons COVID-19 testing: Station B, Zymo kit, 24 samples V.3

Forked from Opentrons COVID-19 testing (Zymo, station B, 48 samples)

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[1]Opentrons Labworks

*In Development* dx.doi.org/10.17504/protocols.io.be47jgzn

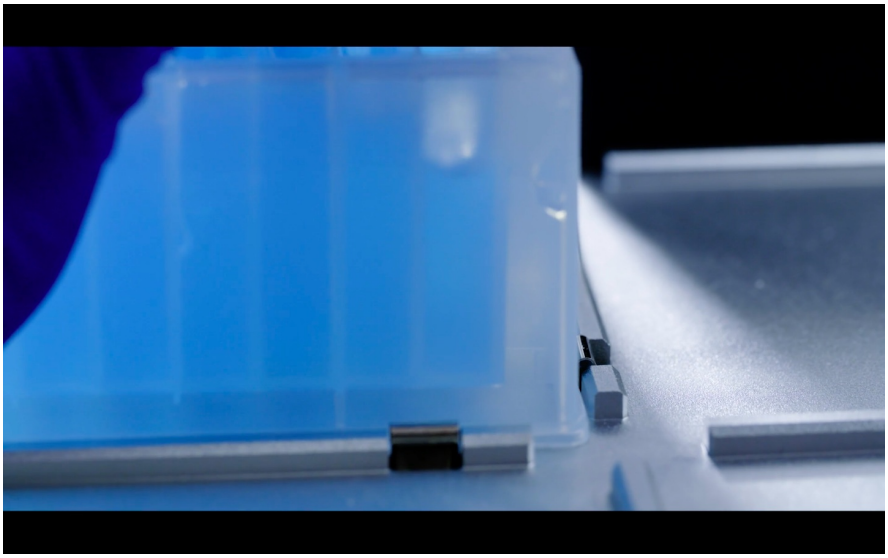
Opentrons COVID-19 Testing

Max Marrone

BEFORE STARTING

When placing labware on the OT-2's deck:

1. Make sure the labware is properly inserted by pressing the corner into the metal springs (pictured below). You should feel a slight click, and the labware should sit completely flat.
2. Make sure the labware is inserted the right way around, with well A1 at the top left.



Closeup of gloved hand pressing reagent trough into the labware holding springs

**Station B**: Initial OT-2 setup

1 Clean the OT-2.

Cleaning an OT-2 COVID-19 Diagnostic Station
**by Max Marrone**

PREVIEW RUN



**Citation:** Max Marrone (04/15/2020). Opentrons COVID-19 testing: Station B, Zymo kit, 24 samples. https://dx.doi.org/10.17504/protocols.io.be47jgzn

1.1 Wipe these parts of the OT-2 down with a 1:10 dilution of bleach:
1. The clear polycarbonate windows.
2. The black pipette stems. (Avoid the rest of the pipettes, including the ejectors.)
3. The aluminum deck.
4. The removable black trash bin.

1.2 Wait **00:00:30** , then quickly rinse the bleach off with distilled water.

The aluminum on the OT-2 will be discolored if the bleach sits for too long. In the long term, it may also cause more serious corrosion.

1.3 Wipe these parts of the OT-2 down with RNaseZap or RNase AWAY.

The same parts that you wiped down with bleach:
1. The clear polycarbonate windows.
2. The black pipette stems. (Avoid the rest of the pipettes, including the ejectors.)
3. The aluminum deck.
4. The removable black trash bin.

Plus these additional parts:
1. The bottoms of the pipette ejectors.
2. Any Temperature Modules or Magnetic Modules that the OT-2 has on its deck.
3. Any 96 well aluminum blocks that are going to be used on the OT-2.

1.4 Rinse the RNaseZap or RNase AWAY off with distilled water.

1.5 Wipe the OT-2 dry, or let the water evaporate.

2 Place the following labware on the OT-2's deck:

**Slot 1:** An Opentrons Temperature Module with an Opentrons 96 well aluminum block and an empty, sterile NEST 100 µL PCR plate.



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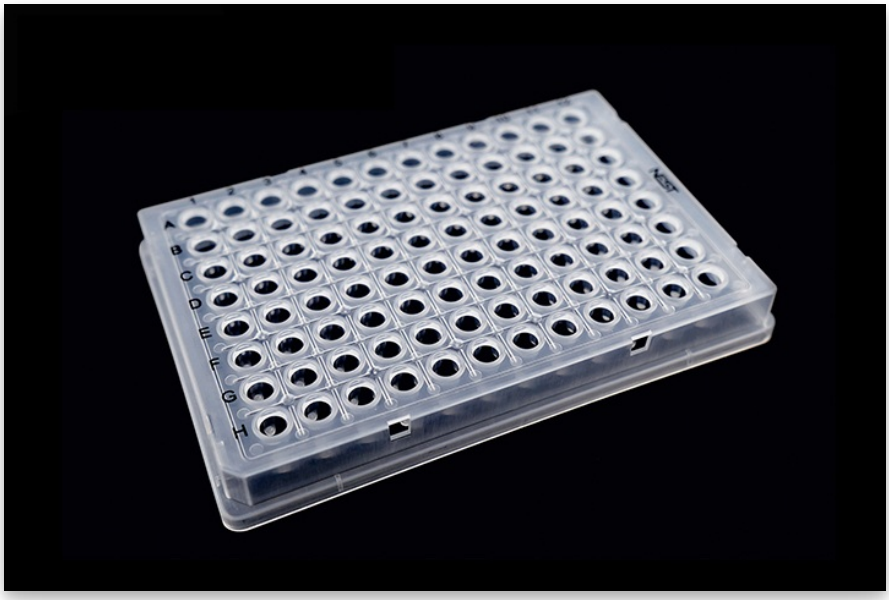

**Slots 3 and 6:** A full, sterile rack of Opentrons 200 µL filter tips.



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**Slot 4:** An Opentrons Magnetic Module with nothing on it.



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**Slot 11:** An empty NEST 1-well reservoir, for organic liquid waste.

3 Start pre-cooling the Temperature Module to **6 °C** .



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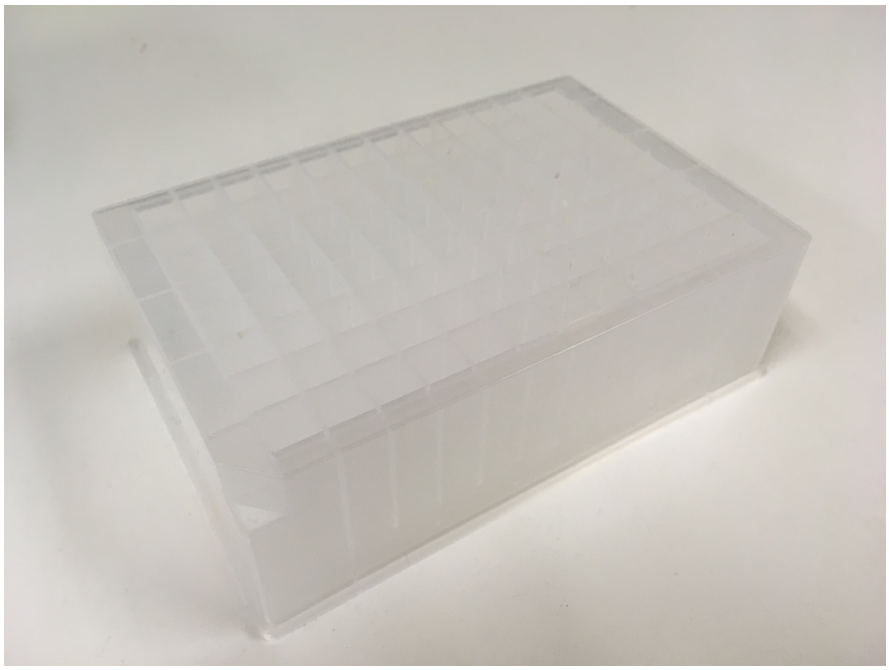
**Station B**: Sample preparation

4 Prepare the **sample well plate.**

The sample well plate is the **NEST 96 deep well plate** that was output from Station A.



The 24 samples (#1–#24) should be laid out like this:

| | Col. 1 | Col. 2 | Col. 3 | Col. 4 | Col. 5 | Col. 6 | Col. 7 | Col. 8 | Col. 9 | Col. 10 | Col. 11 | Col. 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Row A** | #1 | *empty* | #9 | *empty* | #17 | *empty* | *empty* | *empty* | *empty* | *empty* | *empty* | *empty* |
| **Row B** | #2 | *empty* | #10 | *empty* | #18 | *empty* | *empty* | *empty* | *empty* | *empty* | *empty* | *empty* |
| **Row C** | #3 | *empty* | #11 | *empty* | #19 | *empty* | *empty* | *empty* | *empty* | *empty* | *empty* | *empty* |
| **Row D** | #4 | *empty* | #12 | *empty* | #20 | *empty* | *empty* | *empty* | *empty* | *empty* | *empty* | *empty* |
| **Row E** | #5 | *empty* | #13 | *empty* | #21 | *empty* | *empty* | *empty* | *empty* | *empty* | *empty* | *empty* |
| **Row F** | #6 | *empty* | #14 | *empty* | #22 | *empty* | *empty* | *empty* | *empty* | *empty* | *empty* | *empty* |
| **Row G** | #7 | *empty* | #15 | *empty* | #23 | *empty* | *empty* | *empty* | *empty* | *empty* | *empty* | *empty* |
| **Row H** | #8 | *empty* | #16 | *empty* | #24 | *empty* | *empty* | *empty* | *empty* | *empty* | *empty* | *empty* |

Each sample well should already contain a sample, Proteinase K, and internal extraction control RNA, mixed.

**Station B:** Reagent preparation

5 Prepare 3x **14.35 ml Viral DNA/RNA Buffer + MagBinding Beads** ...

5.1 In a sterile 15 mL tube, add **14 ml Viral DNA/RNA Buffer** .



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5.2 Vortex the **MagBinding Beads** in their container.

5.3 Pipette **0.35 ml vortexed MagBinding Beads** to the 15 mL tube.

5.4 Repeat for a total of 3x **14.35 ml Viral DNA/RNA Buffer + MagBinding Beads** .

6 Wait for the Temperature Module to reach **6 °C** .

7 Prepare the **reagent reservoir.**

This step is time-sensitive. The MagBinding Beads will slowly settle in the Viral DNA/RNA Buffer. Continue quickly until you start the run on the OT-2. Vortex the Viral DNA/RNA Buffer + MagBinding Beads immediately before adding them to the reservoir.

Into a **15 mL NEST 12 well reservoir,** pipette or pour the following reagents:

- **Well 1: 14.76 ml Viral DNA/RNA Buffer + MagBinding Beads** (2 prepared tubes' worth)
- **Well 2: 7.38 ml Viral DNA/RNA Buffer + MagBinding Beads** (1 prepared tube's worth)
- **Well 3:** Empty
- **Well 4: 13 ml MagBead DNA/RNA Wash 1**
- **Well 5:** Empty
- **Well 6: 13 ml MagBead DNA/RNA Wash 2**
- **Well 7:** Empty
- **Well 8: 13 ml 95-100% ethanol**
- **Well 9: 13 ml 95-100% ethanol**
- **Well 10:** Empty
- **Well 11:** Empty
- **Well 12: 3 ml nuclease-free water**



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A 15 mL NEST 12 well reservoir.

**Station B:** Final OT-2 setup

8 Place the **sample well plate** in **slot 4**.

9 Place the **reagent reservoir** in **slot 2.**

10 Double-check all the labware to make sure it looks correct.

- Check that labware are inserted the right way around (well A1 at the top-left for the plates, well 1 at the left for the troughs).
- Check that labware are properly clicked into the deck slots.
- Check the deck layout.

**Station B**: Running the robot 3h

11 Run the Station B protocol on the OT-2. The protocol file should be **StationB-24samples-Zymo-20200407.py.**

Operating an OT-2 for COVID-19 testing
**by Max Marrone**
PREVIEW RUN

11.1 Open the Opentrons App.

11.2 Ensure you are connected to the robot. In the **Robots** tab, you can try flipping the robot's lights on and off to test the connection.

11.3 Go to the **Run** tab.

Double-check the name at the top to make sure the correct protocol is uploaded.



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11.4 Click **Start run**. The OT-2 will home its motors and then begin the protocol.

Do not click **Start run** more than once. If you do, a known bug will make the OT-2 run the protocol back-to-back.

**If something goes wrong and you need to abort the protocol:**

1. Shut down the OT-2 with the power switch on its back left side.
2. Turn the OT-2 back on. Wait a couple of minutes for the pipettes to rise.
3. Manually remove any tips attached to the pipettes. (This ensures that the pipettes will not aspirate liquid into themselves when they home.)
4. Reconnect to the OT-2 in the Opentrons App. Click the Home button to move the gantry out of the way so you can access the labware on the deck.

12 Wait for the run to finish.

13 The output is the **NEST 100 µL PCR plate** sitting on the aluminum block atop the Temperature Module in **slot 1.**





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