Opentrons
for Education

Prep Sheet

Serial Dilution

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Opentrons

June 2026

Getting Started

Follow the provided tutorials to set up your OT-2 prior to class.

- ☐ Unboxing the OT-2
- ☐ Set up and import protocols into the Opentrons OT-2 App
- ☐ Attaching and calibrating OT-2 pipettes
- ☐ Completing calibrations on the OT-2
- ☐ Running your first protocol on the OT-2

Additional Support and Resources

OT-2 Instruction Manual
Protocol Designer Instruction Manual

If you need further support, please contact support@opentrons.com. Inform them that you are a part of the Opentrons for Education program and provide the date of your next laboratory class.

If you have questions related to the lesson plan, please reach out to the author, Emily Burghardt, at emily.burghardt@opentrons.com.

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Educator Guide
Serial Dilution

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Contents

Included in this document are the following sections:

- Purpose
- Background Knowledge
- Supplies List
- Experimental Duration
- Basic Troubleshooting
- Procedure Guide

Purpose

This lab guides students through performing a serial dilution, both manually and automated on the Opentrons OT-2 liquid handling robot.

During this lab class, students will learn about and gain experience with:

- Preparing a serial dilution for downstream applications
- Automation on the OT-2 using Protocol Designer
- Comparison of automated and manual pipetting tasks

Core Competencies

Automation Skills:

- Experimental design in protocols for the OT-2
- Automation of pipetting tasks
- Use of the Opentrons OT-2 and no-code protocol tools

Background Knowledge

This lesson plan was prepared for high school students with a basic understanding of common molecular laboratory

methods. *No coding experience is required for this lab.* A pre-lab reading is included in the Student Guide to introduce students to serial dilutions prior to class.

Supplies

Opentrons Equipment

- ☐ Opentrons OT-2 liquid handling robot
- ☐ OT-2 Single or Multi-Channel Pipette

Opentrons Protocol and Tools

- ☐ Opentrons Protocol Designer (must be used in Google Chrome browser)
- ☐ Customizable Serial Dilution for OT-2 protocol (*optional*)

Labware

- ☐ A reservoir like the NEST 12 Well Reservoir 15 mL
- ☐ A well plate like the NEST 96 Well Plate 200 µL Flat

- [ ] OT-2 pipette tips compatible with your single-channel pipette

Reagents

- [ ] Sample to be diluted, like colored water or bacteria in culture media
- [ ] Diluent, like water or culture media

Other

- [ ] Printed Downloadable 96 Well Plate Templates (optional)

Experimental Duration

This lesson plan was prepared for a laboratory class time of 80–90 minutes. Students arrive to class with the OT-2 and reagents set up and ready to use.

To save time, students can perform their serial dilution side-by-side with the Customizable Serial Dilution for OT-2 protocol (rather than creating their own with Protocol Designer).

Basic Troubleshooting and Tips

- We recommend completing a trial run of a simple protocol from the Opentrons Protocol Library prior to class. On an OT-2 robot, a trial run can be a “dry run,” completed without using tips or liquid.

- Issues with tips striking plates are almost always due to using alternate labware or robot calibration issues. If you experience this issue, first confirm that the correct labware specified in the protocol is in use; then, re-calibrate the robot. We always recommend confirming labware offsets with a labware position check before

running any new protocol on the OT-2. This confirms the combination of deck slot and labware definitions the OT-2 will use during the protocol.

- In this lab, students can use Opentrons Protocol Designer to design a serial dilution protocol. Protocol Designer is only supported in Google Chrome. A brief set of instructions are included in the Student Guide. For more, see the Protocol Designer Instruction Manual.

- Instead of using Protocol Designer, students can optionally use the Customizable Serial Dilution for OT-2 protocol. Click **Configure and download** to choose your pipette, tips, reservoir (trough) and plate, dilution factor, number of dilutions, and other details.

- Students can view, test, and edit their protocols using protocol visualization in the Opentrons OT-2 App. They'll be able to see labware and liquid changes for each protocol step, and any errors that they'll need to fix.

Procedure Guide

Before Class

1. Set up the OT-2 robot and the Opentrons OT-2 App by following the tutorials listed in this lesson plan's prep sheet.
2. Prepare samples (we suggest colored water or bacteria samples) and diluent (additional water, or culture media).
3. *Optional:* Configure and download the Customizable Serial Dilution for OT-2 protocol. Customize the protocol with your pipette, tips, reservoir (trough) and plate, dilution factor, and other details.

Lab Introduction

Instructors should define the purpose and real-world uses for a serial dilution, such as:

- Diluting bacterial culture samples for counting
- Drug dosage studies
- Creating a standard curve for an experiment like a BCA assay

As needed, instructors can also demonstrate using Protocol Designer or configuring and downloading a protocol from the

Protocol Library.

Experimental Design- Manual Serial Dilution

1. Students should use a 96-well plate template to design a serial dilution experiment that takes into account the number of samples, transfer and final volumes, and dilutions across the plate.

Experimental Design- Automated Serial Dilution

1. If using the Customizable Serial Dilution for OT-2 protocol, make sure the protocol is configured for the students' serial dilution. Import the protocol into the Opentrons OT-2 App.
2. If using Protocol Designer, allow students time to create a serial dilution protocol. Designing the protocol as a group on a large screen can save time and allow students to work collaboratively. Export the final protocol and import into the Opentrons OT-2 App.
    a. On the protocol's details page, click **Visualize** to check the final protocol for expected behavior.

Manual and Automated Serial Dilutions

1. Guide students in following instructions in the Opentrons OT-2 App to set up the OT-2 for their protocol (whether downloaded or created in Protocol Designer).
2. Match the setup on the OT-2 deck at a laboratory bench.
3. Students can begin their manual serial dilution at the same time as the OT-2. Afterwards, compare and contrast the time each took and the final results.

Discussion Questions

Direct students to discuss today's activities. Example prompts might include:

- What do scientists use a serial dilution for in the lab? Give at least one example discussed in class today.
- Compare and contrast the automated serial dilution (on the OT-2) with your manual serial dilution. What was the time difference? Were there any differences in the final dilution?
- Add your own notes below to define important parts of a serial dilution:

Diluent:

Transfer volume:

Dilution factor:

Final volume:

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Student Guide

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Pre-lab Reading

When scientists work with cells, bacteria, protein, or DNA in the lab, they really work with liquids. Some of these molecular "building blocks," like cells and bacteria, are grown in nutrient-dense liquid called *media.* Protein, DNA or RNA samples might be *suspended*, or mixed, into water or buffer.

Because scientists work with each of these molecular "building blocks" in liquid, knowing the *concentration*, or how much of each is in a certain amount of liquid, is critical. Let's say you're growing bacteria in a culture flask of 50 mL. You know the total amount of liquid, but not the concentration, or how many bacterial cells there are per milliliter of liquid. However, in this case, it could be very difficult to count. There could be several million cells per milliliter of liquid! Here, a scientist might use a *serial dilution.*

A serial dilution is used to progressively dilute your liquid sample several times in a row. Often, this is performed by transferring liquid across the columns of a well plate. Although you'll transfer the same volume each time, the original sample becomes more and more dilute as you move from column 1 to column 12.

To set up a serial dilution, you'll need to consider a few things.

- First, you'll fill the well plate with a *diluent*. This is the liquid used to dilute the original sample.
- Next, transfer your original sample (like bacterial cells) to the first column of the plate. This first column now contains diluent and sample. Mixing the two with your pipette before transferring again can improve your serial dilution.
- Transfer the same volume you just added to the second column of the plate. For example, if you added 5 mL of sample to the first column, you'll transfer 5 mL of the sample + diluent mixture to the second column.
- Repeat the step above for each column of the well plate.

When your serial dilution is complete, you'll have a well plate full of diluted samples. As you move from column 1 to column 12, you move from *least* to *most* dilute, or *highest* to *lowest* concentration of bacterial cells in liquid. If you imagine the same example dilution with colored water, column 1 would be the *darkest* and column 12 would be the *lightest*, as it's the most dilute.

You can use a simple formula to calculate volumes and a *dilution factor* for your own serial dilution:

$$\frac{\text{final volume}}{\text{dilution factor}} = \text{transfer volume}$$

Here, the final volume is the total volume in each well (sample + diluent volume) after each transfer. The transfer volume refers to the volume being transferred from column 1, to 2, to 3, and so on. Your *dilution factor* is the number of times the sample should be diluted across the plate, and it stays the same for each step.

In this lab, you'll compare two serial dilutions: one with an automated protocol, and one serial dilution you'll complete manually!

For help using Protocol Designer, see our Protocol Designer Instruction Manual.

Purpose

In this lab, you'll perform a serial dilution, both manually and automated on the Opentrons OT-2 liquid handling robot. Your instructor will guide you through designing your own serial dilution and automating it with a pre-written or Protocol Designer protocol.

Learning Outcomes

- Prepare a serial dilution for downstream laboratory uses
- Automation on the OT-2 using Protocol Designer
- Comparison of automated and manual pipetting tasks

Supplies

Opentrons Equipment

- ☐ Opentrons OT-2 liquid handling robot
- ☐ OT-2 Single or Multi-Channel Pipette

Opentrons Protocol and Tools

- ☐ Opentrons Protocol Designer (must be used in Google Chrome browser)
- ☐ Customizable Serial Dilution for OT-2 protocol (*optional*)

Labware

- ☐ A reservoir like the NEST 12 Well Reservoir 15 mL
- ☐ A well plate like the NEST 96 Well Plate 200 µL Flat
- ☐ OT-2 pipette tips compatible with your single-channel pipette

Reagents

- ☐ Sample to be diluted, like colored water or bacteria in culture media
- ☐ Diluent, like water or culture media

Other

- ☐ Printed Downloadable 96 Well Plate Templates (*optional*)

Procedure Guide

Before Class

1. Complete the pre-lab reading.

Experimental Design: Manual Serial Dilution

1. Use a 96-well plate template to design a serial dilution experiment. You'll be performing this both manually and with an automated protocol.

   You'll need to choose the number of samples and final volume, and calculate the dilution factor and transfer volumes. *Hint: use the formula in the pre-lab reading.*

Experimental Design: Automated Serial Dilution

1. If your class is using the Customizable Serial Dilution for OT-2 protocol, your instructor can demonstrate configuring and downloading the protocol.
2. If your class is using Protocol Designer to create a serial dilution protocol, you can create your protocol with:
   a. Pipettes (single or multi-channel) and tip racks for the OT-2.

b. A reservoir, like a NEST 12 Well Reservoir 15 mL, containing your diluent.
c. A well plate, like a NEST 96 Well Plate 200 µL Flat, where your serial dilution will take place.
d. Samples, like colored water or bacteria, in the well plate.
e. Transfer steps that transfer diluent + sample across the well plate, either with a single- or multi-channel pipette.

   For more help using Protocol Designer, check out our Protocol Designer Instruction Manual.

3. With your instructor, import the automated protocol into the Opentrons OT-2 App.
   a. On the protocol's details page, click **Visualize** to check the final protocol for expected behavior.

Manual and Automated Serial Dilutions

1. Follow the instructions in the Opentrons OT-2 App to set up the OT-2 for the protocol (downloaded from Protocol Library or created in Protocol Designer).
2. Match the setup you see in the app on the OT-2's deck.
3. Begin your manual serial dilution at the same time as the OT-2! Afterwards, compare and contrast the time each took and the final results.

Discussion Questions

Discuss today's activities with a neighbor.

- What do scientists use a serial dilution for in the lab? Give at least one example discussed in class today.
- Compare and contrast the automated serial dilution (on the OT-2) with your manual serial dilution. What was the time difference? Were there any differences in the final dilution?
- Add your own notes below to define important parts of a serial dilution:

Diluent:

Transfer volume:

Dilution factor:

Final volume: