Opentrons for Education

# Prep Sheet

# Lab Module 2: Automating Laboratory Work with the OT-2

Dr. Cătălin Voiniciuc, Dr. Moni Qiande, and Abigail Lin

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## Getting Started

Review the following resources prior to class.

- [ ] Opentrons Protocol Designer Instruction Manual
- [ ] Running a protocol on the OT-2

## Additional Support and Resources

OT-2 Manual

Opentrons API provides guidelines for writing, customizing, and working with Python protocols for Opentrons robots.

For technical support, please check our Opentrons Help Center for relevant articles. 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 Dr. Cătălin Voiniciuic at cvoiniciuc@ufl.edu.

# Educator Guide

# Lab Module 2: Automating Laboratory Work with the OT-2

Dr. Cătălin Voiniciuc, Dr. Moni Qiande, and Abigail Lin

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## Contents

This educator guide includes the following sections:


- Purpose
- Background Knowledge
- Supplies
- Experimental Duration
- Basic Troubleshooting and Tips
- Procedure Guide
- Discussion Questions

## Purpose

Students will complete a standard curve of the widely used Bradford assay using a BSA standard to quantify protein present in unknown plant tissue samples. In addition, students will compare types of protocols used for the OT-2, gaining experience with:

- Manual and automated pipetting tasks
- Basic viewing and reading of code in Python protocols
- the Bradford BSA assay and protein quantification

### Core Competencies

**Laboratory Skills:**

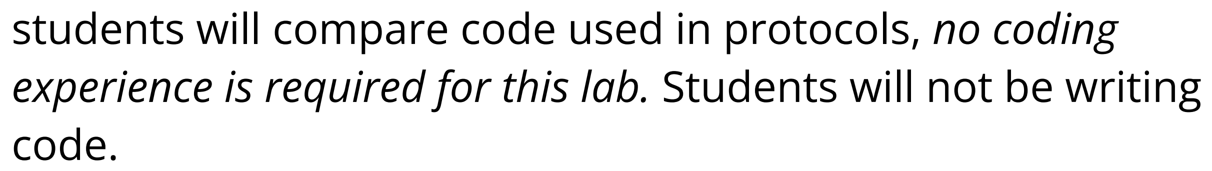
- Following a protocol
- Manual pipetting
- Use of a standard curve

**Automation Skills:**

- Comparison of Python and other Opentrons protocols
- Basic viewing and reading of code

## Background Knowledge

Students should begin this lab with an understanding of the Bradford BSA assay (pre-lab reading materials are included in the Student Guide) and basic pipetting skills. Although



students will compare code used in protocols, *no coding experience is required for this lab.* Students will not be writing code.

## Supplies

### Opentrons Equipment

- ☐ Opentrons OT-2 automated liquid handling robot
- ☐ Opentrons OT-2 P300 Single-Channel GEN2 pipette
- ☐ Opentrons OT-2 P1000 Single-Channel GEN2 pipette
- ☐ Heater-Shaker Module GEN1 (in deck slot 10)

### Opentrons Protocol and Tools

- ☐ Pierce Bradford Protein Assay protocol
- ☐ Opentrons Protocol Library to download an example .JSON file

### Non-Opentrons Equipment

- ☐ Plate reader
- ☐ Benchtop centrifuge

### Labware

- ☐ Corning 96 Well Plate 360 uL Flat on the Universal Flat Adapter and Heater-Shaker Module in deck slot 10
- ☐ NEST 12 Well Reservoir 15 mL in deck slot 5

- ☐ Opentrons 24 Tube Rack with NEST 1.5 mL Snapcap tubes in deck slot 2
- ☐ OT-2 Tips, 300µL in deck slot 4
- ☐ OT-2 Tips, 1000µL in deck slot 9
- ☐ Universal Flat Adapter for Heater-Shaker Module (on the Heater-Shaker Module in deck slot 10)

### Reagents and Other Materials

- ☐ 1.5 mL centrifuge tubes and micropestles for grinding tissue, such as Micro-Pestles with 1.5ml Micro-Tubes
- ☐ Dye reagent concentrate such as Bio-Rad Protein Assay Dye Reagent Concentrate
- ☐ DDI (distilled, deionized water)
- ☐ Whatman #1 filters such as Whatman qualitative filter paper, grade 1
- ☐ Bovine standard albumin (BSA) protein standard at a concentration of 1 mg/mL
- ☐ Plant tissues (used for unknown samples in Bradford assay)
- ☐ 1 centimeter leaf tissue punches
- ☐ *Optional:* liquid nitrogen for tissue grinding

## Experimental Duration

### Required Class Sessions

1

### Lab Run Time

This lesson plan was prepared for a traditional laboratory class time of 80-90 minutes. To save time in class, instructors can optionally prepare plant protein extract from plant tissues ahead of time. Students arrive to class with the dye reagent for the Bradford assay diluted and ready for use.

## Basic Troubleshooting and Tips

- We recommend completing a trial run of the protocol used in this lesson plan prior to class. On the OT-2, this trial run can be completed with or without tips.

- 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.

- The protocol used in this lab uses runtime parameters to customize your experiment. Available parameters include number of samples, sample volume, number of replicates, P1000 single channel mount position, and whether or not a Heater-Shaker Module will be used in the protocol. Runtime parameters can be changed in the Opentrons App prior to running your protocol each time it is used for no-code protocol customization.

- Labware, liquids, and setup instructions included in this lesson plan are written based on a specific set of runtime parameters (see **Bradford Assay- Standard Curve** in the **Procedure Guide**). Changes to labware, liquids, and deck setup may occur when different runtime parameters are chosen, and will be visible in the Opentrons App.

- In the protocol used in this lesson plan, the OT-2 will pipette each protein sample (from protein standard series or unknown samples) into individual wells of the working plate. Remind students that any liquid definitions in the protocol communicate to the OT-2 the identity of liquids in each well. However, students should be responsible for knowing which samples are in which wells. The “run preview” in the Opentrons App (generated during setup, after selection of runtime parameters) can be used by students to create a “plate map” and keep track of their samples. This is also an opportunity for

students to become familiar with the run preview and other app features.

- Absorbance measurements should be taken relatively quickly after the incubation period. Absorbance will increase over time, and samples should incubate for no longer than 1 hour.

## Procedure Guide

### Before Class

1. Dilute 1 part of dye reagent concentrate with 4 parts of DDI water.
2. Filter through a Whatman #1 filter (or equivalent) to remove particulates. Diluted reagent may be used for approximately 2 weeks when stored at room temperature and away from light.
3. *Optional:* to save class time, plant protein extractions may also be completed prior to the lab.
4. Choose two demonstration protocols from the Protocol Library (one Python (.py) file and one .json file). After searching for protocols of interest, the “editability” filter on the left hand side can be used to find .py files (editability- Python) and .json files (editability- Protocol Designer). Download and import protocols into the Opentrons App.

Download the Pierce Bradford Protein Assay protocol and import into the Opentrons App. A test run may be helpful before class.

### Lab Introduction

1. Instructors may want to provide a brief overview of the Python programming language and review the use of the Opentrons Protocol Designer in the previous module. A beginner-friendly overview can be found in the official Python beginner's guide: Beginners Guide Overview - Python Wiki.
2. Instructor should introduce methods of giving the OT-2 commands:
    - *a.* *How does the OT-2 know what to do*? The OT-2 must communicate with, and receive directions from a computer in the form of commands. A protocol is a list of commands the OT-2 will complete. Each is a liquid handling task. *Note: students should understand both uses of the word "protocol:" as a written experimental process for scientists, and instructions for the robot in the form of a protocol file.*
    - *b.* The first method: a ready-made protocol from the Protocol Library (students are familiar with this from the previous module). These protocols can be customized using *parameters* (values chosen by the

user and added into the protocol). Protocol Library protocols can be either Python (.py) or .json files.
c. The second method: creating your own protocol as a user, either by writing code (in your own Python protocol) or using the no-code Opentrons Protocol Designer to generate a .json file.

**How to give the OT-2 commands: protocol type comparison**

1. Open the Python protocol file downloaded from the Protocol Library. Have students view a large screen display for comparison. Show students the Python protocol, pointing out the various parts that detail the protocol and the directions:
    a. *Metadata (robot type, author)*- descriptions of the protocol
    b. *load_labware*- describes to the robot the labware that is present on the deck
    c. *Tasks for OT-2 to complete:* commands like aspirating and dispensing for a volume transfer

2. Open the .json protocol file from the Protocol Library so that students are able to read the code. Although both protocols use code to direct the OT-2, and the robot can easily decipher both, one is easier for us to read as humans (note differences in structure). *Note:* protocols can be imported in the Opentrons App as well, to compare and contrast the protocol steps in a graphic interface with the code students will also see.

### Plant Protein Extraction

1. Students should use leaf tissue punches to add small amounts of their selected tissues to 1.5 mL centrifuge tubes.
2. Plant tissue can be ground into a fine powder in the tubes using the micropestles. Liquid nitrogen can help to grind the tissue, but is optional.
3. Students should add 300 μL of DDI water and grind any remaining plant tissue fragments.
4. Centrifuge the tubes in a benchtop centrifuge at >16,000 xg for 2 minutes.

5. Transfer the supernatant. An unknown protein series will be prepared in the **Bradford Assay- Unknown Samples** section.

## Bradford Assay- Standard Curve

1. Open the Pierce Bradford Protein Assay protocol in the Opentrons App.
2. Students should prepare a series of protein standards (1 mg/mL BSA) with the pipetting scheme shown below:

| **BSA 1 mg/mL)** | 0 | 1 | 2 | 4 | 8 | 10 |
|---|---|---|---|---|---|---|
| **DDI water** | 10 | 9 | 8 | 6 | 2 | 0 |
| **Total** | 10 | 10 | 10 | 10 | 10 | 10 |

The six samples shown here should be loaded into the NEST 1.5 mL snapcap tubes in the Opentrons tube rack that will be placed on the deck.

The protocol setup instructions below are written for a protocol with 6 samples. Using runtime parameters, your protocol can be customized in the Opentrons App for up to 48 samples. Note changes to labware, liquids, and deck

setup when customizing your protocol with runtime parameters.

3. Start setup for the protocol on your OT-2 to customize your protocol with runtime parameters. The following labware, liquids, and setup steps are written according to the example runtime parameters shown below.
    a. Number of samples (enter your value 1-48; for example: *6 samples)*
    b. Sample volume (choose 10 or 150 µL; for example: *10 µL)*
    c. Number of replicates (enter your value 1-3; for example: *3 replicates*)
    d. P1000 single-channel pipette position (choose the left or right mount; for example: *left mount)*
    e. Heater/Shaker on deck (choose yes or no; for example: *yes)*
4. Guide students to set up labware and liquids on the OT-2's deck based on the chosen parameters (shown in the "Labware" and "Liquids" sections during setup).
    a. Corning 96 Well Plate 360 µL Flat, also known as the *working plate;* on the universal flat adapter on the Heater-Shaker Module in deck slot 10
    b. NEST 12 Well Reservoir 15 mL in deck slot 5, with 7 mL of *working reagent* (dye reagent) in well A1
    c. Opentrons 24 Tube Rack with NEST 1.5 mL snapcap tubes in deck slot 2

        i. Wells A1-B2 each contain 40 µL of sample per tube
    d. OT-2 96 Tip Rack 1000 µL in deck slot 9
    e. OT-2 96 Tip Rack 300 µL in deck slot 4

5. Follow the Opentrons App to confirm hardware, labware and liquid locations, and initial volumes. Run the protocol.
6. The OT-2 will transfer samples to wells of the *working plate* (enough for 3 replicates of each sample). The OT-2 will first mix, then transfer each sample to three wells of the working plate, starting with well A1.
7. The OT-2 will then transfer the *working reagent* to each well of the working plate containing sample. The OT-2 will first mix, then transfer dye reagent to each well.
8. The OT-2 will close the latch of the Heater-Shaker Module to secure the working plate in place.
9. The OT-2 will mix the sample and working reagent thoroughly at 1250 RPM for 30 seconds.
10. The OT-2 will open the latch of the Heater-Shaker Module and pause to allow sample incubation for 10 minutes.
11. Remove plate from Heater-Shaker Module manually.
12. Students will manually measure absorbance at 595 nm using a plate reader. Remind students to begin by taking appropriate control measurements (water).

Students will plot the standard curve of the BSA and/or IgG standards. A sample standard curve is included in the Student Guide.

### Bradford Assay- Unknown Samples

1. Students will use aliquots from a plant protein extraction as unknown samples (either prepared fresh in class as above in the “Plant Protein Extraction” section, or prepared before class).
2. Students should again prepare a protein series, this time with unknown plant protein samples, according to the pipetting scheme shown below:

| **Unknown sample:** | 10 | 10 | 10 | 10 | 10 | 10 |
|---|---|---|---|---|---|---|
| **Total** | 10 | 10 | 10 | 10 | 10 | 10 |

3. Set up sufficient labware and liquids to run the protocol a second time.
4. Check your chosen runtime parameters and run the protocol.
5. The OT-2 will transfer samples to wells of the *working plate* (enough for 3 replicates of each sample). The OT-2

will first mix, then transfer each sample to three wells of the working plate, starting with well A1.

6. The OT-2 will then transfer the *working reagent* to each well of the working plate containing sample. The OT-2 will first mix, then transfer dye reagent to each well.
7. The OT-2 will close the latch of the Heater-Shaker Module to secure the working plate in place.
8. The OT-2 will mix the sample and working reagent thoroughly at 1250 RPM for 30 seconds.
9. The OT-2 will open the latch of the Heater-Shaker Module and pause to allow sample incubation for 10 minutes.
10. Remove plate from Heater-Shaker Module manually.
11. Students will manually measure absorbance at 595 nm using a plate reader. Remind students to begin by taking appropriate control measurements (water, and extraction buffer or solvent ground plant tissues are suspended in).
12. Students will use their standard curve to calculate the amount of protein in the unknown samples.

## Discussion Questions

Direct students to discuss the lab activities with one another. Example prompts might include:

- How does the OT-2 know what to do? List the methods we discussed in class. Provide one advantage and one disadvantage for each.
- Compare your standard curve and unknown protein calculations with a neighbor. Are there differences? Why might that be?
- How did your pipetting compare to the OT-2 today? Were there advantages or disadvantages to either method?

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Student Guide

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## Pre-Lab Reading

What is in a cell? In other classes, you may have learned about *organelles*, structures within a plant cell that each do a specific job, like the nucleus, mitochondria, and chloroplasts, among many others. If we looked a little deeper into the cell, we would also find lots of proteins.

A protein is a molecule that is made up of amino acids. That's a fairly simple definition, because there are many classes of proteins that can serve a wide variety of functions. Enzymes are proteins that allow biological reactions to occur. Some hormones, like insulin, that send signals among the organs in your body, are proteins. Proteins are also important for structural support, such as in the *cytoskeleton,* a structure inside the cell required for movement and cell division… and these are just a few functions of proteins! In fact, if we consider the components of a cell by weight alone, proteins make up about half of the dry mass. For all these reasons, scientists around the world study proteins and their functions.

How do scientists know how much protein is in a cell? One method, which we will use today, is called the Bradford assay. This assay relies on the binding of a colored dye to proteins in a solution. When the dye binds to protein, a shift in the *absorbance* maximum occurs. Absorbance, which we will measure in this lab as *optical density*, is the amount of light

absorbed by a solution. The more light that is absorbed by a solution, the less light there is that is allowed to pass through the solution. More specifically, when the dye binds to proteins, the absorbance maximum will shift from a wavelength of 465 nm to 595 nm. So: before protein binding, there would be no absorbance measured at 465 nm; after protein binding, there will be some amount of absorbance that we can measure at 595 nm.

So far, we will be able to tell *if* there is protein in the solution- how will we know the *quantity?* To do this, you will prepare a standard curve using a protein standard- a solution containing proteins that we already know the concentration of. You will then measure the absorbance of a solution with an unknown concentration of protein, and compare it to your known concentration (and the absorbance measurement associated with it).

This assay will require pipetting of both the protein solutions and the dye, so we can use the OT-2 to automate this experiment. However, today we will do a bit of comparison- how does your pipetting for this assay compare to the OT-2?

You can read more about the Bradford assay using a BSA protein standard here: Bio-Rad Protein Assay.

## Purpose

In this lab, you will complete a standard curve of the widely used Bradford assay using BSA standards, and quantify protein present in unknown plant tissue samples. In addition, we'll compare types of programming languages for protocols used on the OT-2.

### Learning Outcomes

- Compare manual and automated pipetting tasks
- Understand the differences between protocol "methods" and compare Python and Protocol Designer protocols
- Graph a standard curve based on absorbance measurements of protein standards
- Calculate amounts of protein in unknown samples using standard curve
- Understand the application of the Bradford assay for protein quantification

## Supplies

### Opentrons Equipment

- [ ] Opentrons OT-2 automated liquid handling robot
- [ ] Opentrons OT-2 P300 Single-Channel GEN2 pipette
- [ ] Opentrons OT-2 P1000 Single-Channel GEN2 pipette

- ☐ Heater-Shaker Module GEN1 (in deck slot 10)

### Opentrons Protocol and Tools

- ☐ Pierce Bradford Protein Assay protocol
- ☐ Opentrons Protocol Library to download an example .JSON file

### Non-Opentrons Equipment

- ☐ Plate reader
- ☐ Benchtop centrifuge

### Labware

- ☐ Corning 96 Well Plate 360 uL Flat on the Universal Flat Adapter and Heater-Shaker Module in deck slot 10
- ☐ NEST 12 Well Reservoir 15 mL in deck slot 5
- ☐ Opentrons 24 Tube Rack with NEST 1.5 mL Snapcap tubes in deck slot 2
- ☐ OT-2 Tips, 300µL in deck slot 4
- ☐ OT-2 Tips, 1000µL in deck slot 9
- ☐ Universal Flat Adapter for Heater-Shaker Module (on the Heater-Shaker Module in deck slot 10)

### Reagents and Other Materials

- ☐ 1.5 mL centrifuge tubes and micropestles for grinding tissue, such as Micro-Pestles with 1.5ml Micro-Tubes
- ☐ Dye reagent concentrate such as Bio-Rad Protein Assay Dye Reagent Concentrate
- ☐ DDI (distilled, deionized water)
- ☐ Whatman #1 filters such as Whatman qualitative filter paper, grade 1
- ☐ Bovine standard albumin (BSA) protein standard at a concentration of 1 mg/mL
- ☐ Plant tissues (used for unknown samples in Bradford assay)
- ☐ 1 centimeter leaf tissue punches
- ☐ *Optional:* liquid nitrogen for tissue grinding

## Procedure Guide

### Before Class

1. Complete the pre-lab reading.

### How to give the OT-2 commands: protocol type comparison

1. Your instructor will demonstrate the different types of protocols that can be used to provide commands to the OT-2. See the table below, and make your own notes.

| Protocol file extension | Where to find it? | How to customize |
| --- | --- | --- |
| .json | Opentrons Protocol Designer, and some Protocol Library protocols | No-code protocols can be built in Protocol Designer.<br><br>Code can be reformatted and read, but has less structure. |
| .py | Most Protocol Library protocols, or write your own in Python! | Data can be added from a CSV file or lines of code can be edited. |

Remember that, in automation of lab tasks, the word "protocol" has two meanings: protocols are a written experimental process for scientists to follow, as well as instructions for the robot (as a protocol file).

### Plant Protein Extraction

1. Use leaf tissue punches to add small amounts of your selected tissue to a 1.5 mL centrifuge tube.
2. Use a micropestle to grind the tissue into a fine powder. Liquid nitrogen can help to grind the tissue, but is optional.

3. Add 300 µL of DDI water and grind any remaining plant tissue fragments.
4. Centrifuge your centrifuge tube in a benchtop centrifuge at >16,000 xg for 2 minutes.
5. Transfer supernatant to a new tube.

### Bradford Assay- Standard Curve

1. Open the [Pierce Bradford Protein Assay ]protocol in the Opentrons App.
2. Prepare a series of protein standards (1 mg/mL BSA) using the pipetting scheme below:

| **BSA 1 mg/mL)** | 0 | 1 | 2 | 4 | 8 | 10 |
|---|---|---|---|---|---|---|
| **DDI water** | 10 | 9 | 8 | 6 | 2 | 0 |
| **Total** | 10 | 10 | 10 | 10 | 10 | 10 |

The six samples shown here should be loaded into the NEST 1.5 mL snapcap tubes in the Opentrons tube rack that will be placed on the deck.

The protocol setup instructions below are written for a protocol with 6 samples. Using *runtime parameters,* you can customize this protocol in the Opentrons App each time you run it. If your class needs a different number of samples, be

sure to check the app for changes to the labware, liquids, and deck setup for your protocol.

3. Start setup for the protocol on your OT-2 to customize your protocol with runtime parameters. The following labware, liquids, and setup steps are written according to the example runtime parameters shown below.
    a. Number of samples (enter your value 1-48; for example: *6 samples)*
    b. Sample volume (choose 10 or 150 µL; for example: *10 µL)*
    c. Number of replicates (enter your value 1-3; for example: *3 replicates*)
    d. P1000 single-channel pipette position (choose the left or right mount; for example: *left mount)*
    e. Heater/Shaker on deck (choose yes or no; for example: *yes)*
4. Set up your labware and liquids on the deck of the OT-2 based on the chosen parameters (shown in the “Labware” and “Liquids” sections during setup).
    a. Corning 96 Well Plate 360 µL Flat, also known as the *working plate;* on the universal flat adapter on the Heater-Shaker Module in deck slot 1
    b. NEST 12 Well Reservoir 15 mL in deck slot 5, with 7 mL of *working reagent* (dye reagent) in well A1
    c. Opentrons 24 Tube Rack with NEST 1.5 mL snapcap tubes in deck slot 2

i. Wells A1-B2 each contain 40 µL of sample per tube

d. OT-2 96 Tip Rack 1000 µL in deck slot 9

e. OT-2 96 Tip Rack 300 µL in deck slot 4

5. Follow the instructions in the Opentrons App to confirm the hardware, labware and liquid locations, and initial volumes. Run the protocol.
6. The OT-2 will transfer samples to wells of the *working plate* (enough for 3 replicates of each sample). The OT-2 will first mix, then transfer each sample to three wells of the working plate, starting with well A1.
7. The OT-2 will then transfer the *working reagent* to each well of the working plate containing sample. The OT-2 will first mix, then transfer dye reagent to each well.
8. The OT-2 will close the latch of the Heater-Shaker Module to secure the working plate in place.
9. The OT-2 will mix the sample and working reagent thoroughly at 1250 RPM for 30 seconds.
10. The OT-2 will open the latch of the Heater-Shaker Module and pause to allow sample incubation for 10 minutes.
11. Remove plate from Heater-Shaker Module manually.
12. Measure the absorbance at 595 nm using a plate reader. Be sure to complete your control measurements first (for example, a plate filled with water, and a plate filled with the extraction buffer or solvent that your plant unknown samples are diluted in).

13. Using the absorbance (measured as *optical density)* and protein concentration in each well, plot your standard curve. An example is shown below:

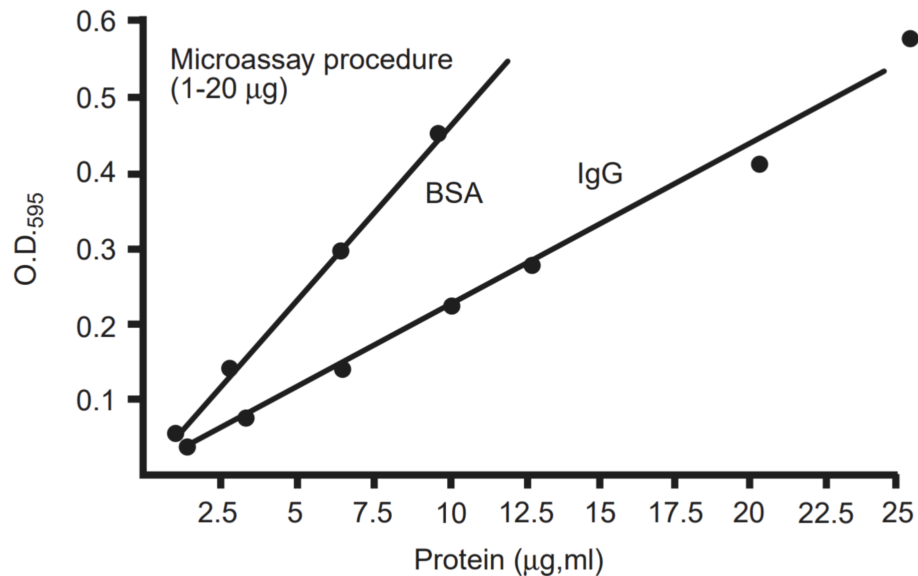
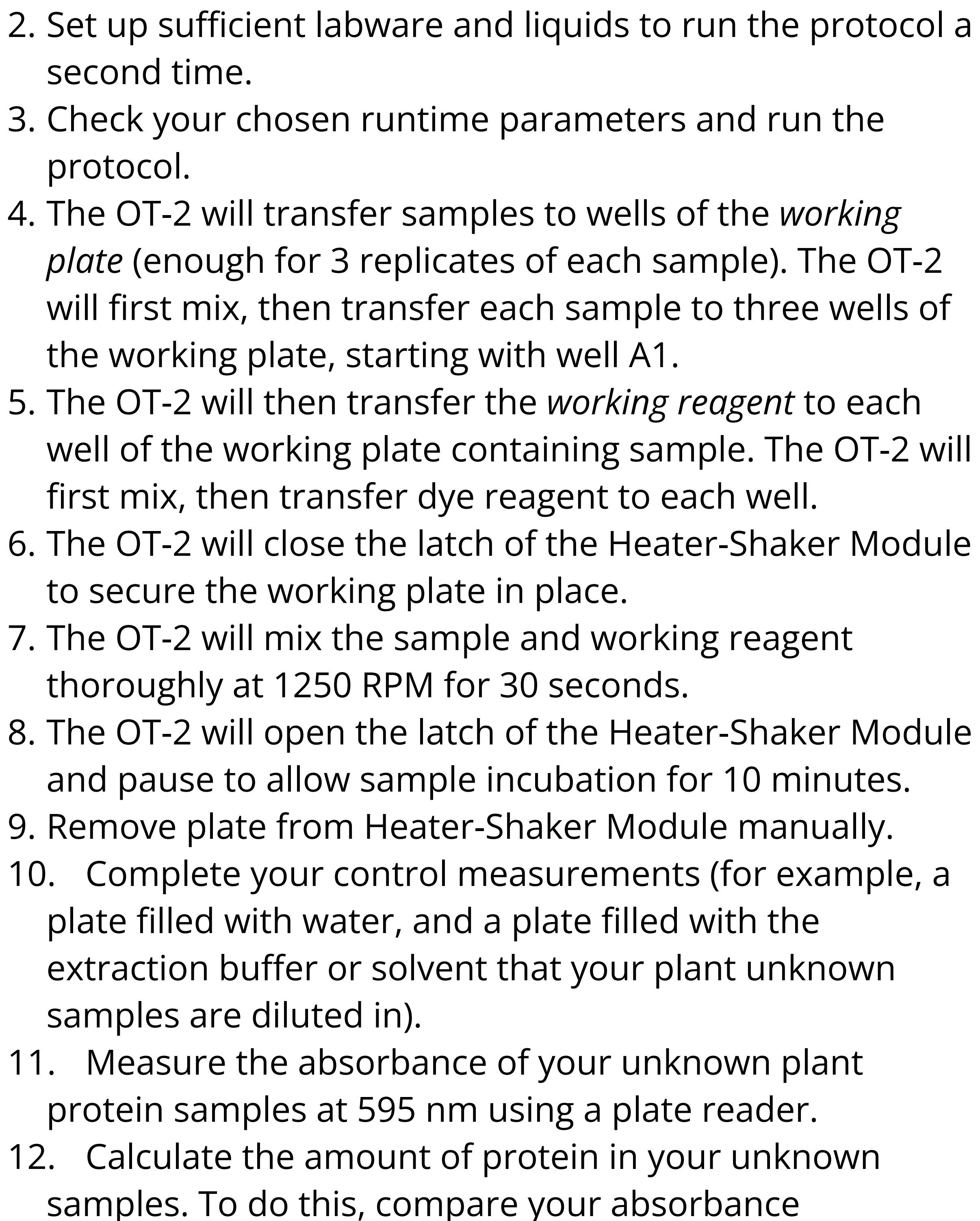



**Fig. 2. Typical standard curve for the Bio-Rad Protein Microassay (1-20 µg/ml), bovine gamma globulin (standard I), bovine serum albumin (standard II).**
$O.D._{595}$ corrected for blank. 1.25-25 µg/ml x 0.8 ml = 1-20 µg protein.

## Bradford Assay- Unknown Samples

1. Prepare a second protein series for the assay, this time pipetting your unknown protein samples according to the table below.

| **Unknown sample:** | 10 | 10 | 10 | 10 | 10 | 10 |
|---|---|---|---|---|---|---|
| **Total** | 10 | 10 | 10 | 10 | 10 | 10 |



2. Set up sufficient labware and liquids to run the protocol a second time.
3. Check your chosen runtime parameters and run the protocol.
4. The OT-2 will transfer samples to wells of the *working plate* (enough for 3 replicates of each sample). The OT-2 will first mix, then transfer each sample to three wells of the working plate, starting with well A1.
5. The OT-2 will then transfer the *working reagent* to each well of the working plate containing sample. The OT-2 will first mix, then transfer dye reagent to each well.
6. The OT-2 will close the latch of the Heater-Shaker Module to secure the working plate in place.
7. The OT-2 will mix the sample and working reagent thoroughly at 1250 RPM for 30 seconds.
8. The OT-2 will open the latch of the Heater-Shaker Module and pause to allow sample incubation for 10 minutes.
9. Remove plate from Heater-Shaker Module manually.
10. Complete your control measurements (for example, a plate filled with water, and a plate filled with the extraction buffer or solvent that your plant unknown samples are diluted in).
11. Measure the absorbance of your unknown plant protein samples at 595 nm using a plate reader.
12. Calculate the amount of protein in your unknown samples. To do this, compare your absorbance

measurements. What was the protein concentration of the standard sample that most closely matches the absorbance measurement of your unknown sample?

## Discussion Questions

Turn to a neighbor and discuss.

- How does the OT-2 know what to do? List the methods we discussed in class. Provide one advantage and one disadvantage for each.
- Compare your standard curve and unknown protein calculations with a neighbor. Are there differences? Why might that be?
- How did your pipetting compare to the OT-2 today? Were there advantages or disadvantages to either method?