Biomanufacturing Kit Module 3 Syllabus

Please Note: These labs are designed to be flexible. All documents are provided as '.doc' or '.ppt' so that teachers can download them and modify them to suit their curriculum, classroom, student population etc

Video link: Amgen – How to purify RFP

https://www.youtube.com/watch?v=AkllY9_4rhA

Downstream Process

1. Preparation of Bacterial Lysate : Module 3, Lesson 1
    a. Review Downstream Process: Downstream Process slide deck
    b. Each team prepares a bacterial lysate sample from their harvested bacterial culture: Bacterial Lysis Protocol
    c. Each team fills out the appropriate parts of the Downstream Process Batch Record.
2. Protein Purification: Module 3, Lesson 2
    a. Prepare the Column: Column Purification Protocol
    b. Prepare the bacterial lysate to be loaded on the column
    c. Purify RFP or GFP from the bacterial lysate using column chromatography
    d. Each team fills out the appropriate parts of the Downstream Process Batch Record document
3. Protein Concentration Standard Curve: Module 3, Lesson 3
    a. Bradford Protein Assay slide deck
    b. Process Engineers prepare albumin standard curve samples: Bradford Protein Assay Standard Curve Protocol
    c. The Process Engineers prepare the spectrophotometer for use
    d. The Process Engineers take OD595 readings of the standard curve samples
    e. The Process Engineers record the data in the Protein Concentration Standard Curve Report
    f. The Protein Concentration Standard Curve Report is made available to all teams.

    NOTE: It may work well to have the members of each team work on Module 3, Lesson 4 while the Process Engineers are preparing the spectrophotometer and creating the standard curve samples.
4. Measurement of Protein Concentration of Purified Protein: Module 3, Lesson 4
    a. Each team heat denatures an aliquot of their purified protein
    b. The QC Technician prepares an aliquot of the team's heat denatured purified protein for the Bradford assay
    c. The QC Technician reads the OD595 of the sample
    d. Each team fills out the appropriate part of the Downstream Process Batch Record.

5. Calculation of Protein Concentration and Yield: Module 3, Lesson 5
    a. Each team plots a standard curve and finds the equation of the best fit line
    b. Each team uses the equation of the line to calculate the concentration of their purified protein.
    c. Each team calculates their protein yield.
    d. Each team fills out the appropriate part of the Downstream Process Batch Record.
6. Creation of a product label
    a. Teams create a product name
    b. Teams create a product label

Biomanufacturing Module Three: Downstream Process

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	Topic / Activity	Summary
Day 1	Review of Downstream Process, Preparation of bacterial lysate ● Spin down 2 x 1mL of bacterial culture ● Remove supernatant ● Resuspend bacterial pellets and lyse overnight	On day one, teams review the Downstream Process. The Downstream Technicians prepare a bacterial lysate.
Day 2	Harvest bacterial proteins, Purify RFP (or GFP) ● Spin down lysate ● Harvest protein-containing supernatant ● Purify using column chromatography	On day two, student teams will purify their RFP or GFP. The Downstream technicians from each team will purify the protein over a column.
Day 3	Create protein concentration standard curve with albumin ● Run Bradford assay to measure protein concentration ● Set up standard curve with albumin protein ● Read the OD595 with the spectrophotometer	On day three, the Process Engineers from each team prepare standard curve samples for the whole class. The Process Engineers calibrate the spectrophotometer. The Process Engineers read the samples on the spectrophotometer and record the data. **NOTE:** It may work well to have the rest of the team members carry out the Day 4 activities while the Process Engineers create the standard curve samples.
Day 3 or 4	Measure protein concentration of purified RFP (or GFP) ● Denature purified protein with heat ● Run Bradford assay to measure protein concentration ● Set up sample tube - purified protein ● Read the OD595	On day four, Downstream Technicians prepare aliquots of purified protein and bacterial lysate for the Bradford assay. The Process Engineer calibrates the spectrophotometer. The QC Technician reads the sample on the spectrophotometer and records the data.
Day 5	Calculation of protein concentration, Batch record completion	On Day 5 each team graphs the standard curve samples using the data

		collected by the Process Engineers. Student teams determine the concentration of their purified protein using a graph of the standard curve. The teams calculate their total purified protein yield. The QC Technicians fill out the purified protein batch record. The QA Technicians sign off on the completed batch records and file them.
Day 6	Vial filling and labeling	On Day 6 teams create a label for their protein product.

Biomanufacturing Module 3

Lesson 1 – Bacterial Lysis

Lesson objectives:

Students will understand:

- How to perform a bacterial lysis.

Essential Question

- Why do you need to lyse the RFP+ (or GFP+) bacteria?

Materials:

- Intro to Downstream Process - Protein Purification slide deck
- Bacterial Lysis Protocol
- p1000 micropipette and tips (1/team)
- 1.5 mL microfuge tubes (2/team)
- Microcentrifuge (1/class)
- p200 micropipette and tips (1/team)
- Elution buffer (TE) (500ul/team)
- Lysis Buffer (BPER) (500ul/team)
- 10% bleach solution in a beaker - sitting in the sink or inside another container (1/team)
- Downstream Process Batch Record Document

What Students Will Do

- Listen to/watch the Intro to Downstream Process - Protein Purification slide deck
- Mix their harvested RFP+ or GFP+ bacteria.
- Follow the Bacterial Lysis protocol
- Each team fills out the appropriate parts of their Downstream Process Batch Record

Teacher Preparation

- Prior to class make copies of
    - Bacterial Lysis Protocol (one per team)
    - Downstream Process Batch Record Document (one per team)
- Prior to class prepare 500uL aliquots of Elution Buffer (TE) (one per team).
- Prior to class prepare 500uL aliquots of Lysis Buffer (BPER) (one per team).
- Prior to class prepare beakers half full of 10% bleach solution (one per team). Put these in the sink or inside of another container - to contain spills
- Provide each team
    - One p1000 micropipette and tips
    - One p200 micropipette and tips
    - Two 1.5mL microfuge tubes
    - Sharpie marker for labeling tubes
    - One 500uL aliquot of Elution buffer (TE)
    - One 500uL aliquot of Lysis buffer (BPER)
    - Spray bottle of 70% ethanol
    - Paper towels

- Team file folders

Organizer

Time	Activity	Materials
15 minutes	Present the Intro to Downstream Process - Protein Purification slide deck	Slide deck
5 minutes	Members of all teams put on PPE	Lab coats, gloves, safety goggles
5 minutes	Teams sanitize and prepare their bench space	70% ethanol, paper towels, micropipettes, tips, harvested bacteria
15 minutes	The Downstream Process Technicians from each team carry out the bacterial lysis protocol.	Bacterial Lysis Protocol,1.5mL microfuge tubes, tube rack, micropipettes, tips, Elution buffer, Lysis buffer
5 minutes	Teams label their bacterial lysate tubes and place them in a tube rack to incubate at room temperature overnight. Tubes containing harvested bacteria get stored in the refrigerator.	Sharpie markers Tube rack
10 minutes	Teams fill out the appropriate portions of their Downstream Process Batch Record Document and file it	Upstream Process Batch Record Document, Team File Folder

Procedure

Introduction to Protein Production in Bacteria

1. Present the Introduction to Downstream Process - Protein Purification slide deck.

Preparation of the bacterial lysate

2. Members of each team put on PPE
3. Each team sanitizes and organizes their bench space
4. The Downstream Process Technicians from each team follow the Bacterial Lysate Protocol to create a bacterial lysate.
5. Each team puts their tubes containing the bacterial lysate in a tube rack to incubate at room temperature overnight.
6. Each team fills out the appropriate sections of the Downstream Process Batch Record and files it in their team file.

Downstream Process - Bacterial Lysis Protocol

Materials:

1. **Harvested RFP+ or GFP+ Bacterial Culture:** This culture contains the bacteria you need to lyse (break open) so you can purify the RFP or GFP.
2. **Micropipette tips**: For measuring small volumes of reagents or bacterial cultures
3. **Microfuge tubes of various sizes:** For containing small volumes of reagents or bacterial cultures
4. **Microfuge tube rack**: To hold microfuge tubes.
5. **Biohazard waste container**: This is the container you will put all solid bacterial waste into.
6. **10% bleach solution**: This is the solution you will put all liquid bacterial waste into.
7. **Sharpie markers**: For correct labeling of samples.
8. **Elution Buffer (TE)**: This is a buffered solution you will resuspend your bacteria in prior to lysis.
9. **Lysis Buffer (BPER)**: This is the solution you will use to break open bacterial cells.
10. **Downstream Process Batch Record Form**: When properly filled out, this form is a record of your entire upstream process.

Equipment:

1. **Microcentrifuge**
2. **Micropipettes (p1000 and p200)**

Protocol:

Done by the Downstream Process Technician

The Downstream Process Technician should work with their team members to carry out this procedure.

Dispose of all waste properly.

You are STRONGLY ENCOURAGED to check off each step below as you complete it.

1. Examine your harvested bacterial culture and record its color.

2. Before you can lyse the bacterial cells, you will need to separate them from the growth medium.

3. Make sure the cap on the 50mL tube containing your team's harvested bacteria is tightly screwed on. The tube has been stored in the refrigerator and the bacteria have settled at the bottom of the tube. Mix the tube well by inverting it repeatedly until the mixture is evenly mixed.

4. Set a p1000 micropipette to 500uL. Place a clean tip on the micropipette and use it to move 1mL of the bacterial mixture into a 1.5mL microfuge tube. Discard the tip into the biohazard waste container when you are through.

5. Repeat step 4 with a second microfuge tube.

6. Label both tubes with your Team name and the date. You will only use one of these tubes initially. The second tube will be saved as a 'back up'.

7. Spin the tubes in a microcentrifuge set at 5,000 rpm for 3 minutes.
   NOTE: Be sure to balance the tubes in the microcentrifuge before spinning.

8. Carefully remove the tubes from the microcentrifuge. You should be able to see a bacterial pellet at the bottom of each tube.

9. Set a p1000 micropipette to 300uL. Place a fresh tip on the micropipette and carefully remove the supernatant (liquid) from the tubes without disturbing the cell pellet. The liquid can be dispensed into the 10% bleach solution.

10. Using a p200 micropipette, add 150uL of Elution Buffer to each cell pellet.

11. Close the tube caps and drag the tubes vigorously across the surface of a microfuge tube rack to mix. You can also pipette up and down with the micropipette if you prefer. Mix until you no longer see clumps of bacterial cells.

12. Using the p200 micropipette, add 150uL of Lysis Buffer (BPER) to each tube.

13. Close the tube caps and drag the tubes vigorously across the surface of a microfuge tube rack to mix. You can also pipette up and down with the micropipette if you prefer.

14. Label each tube: Lysate + team name + date.

15. The tube will incubate overnight at room temperature.

16. Place all used tips and tubes into the biohazard container.

17. Place your harvested bacteria back into the refrigerator.

18. Fill out the appropriate parts of the Downstream Process Batch Record.

Biomanufacturing Module 3

Lesson 2 – Protein Purification

Lesson objectives:

Students will understand:

- How to perform protein purification using column chromatography.

Essential Question

- Why does the protein need to be purified?

Materials:

- Intro to Downstream Process - Protein Purification slide deck
- Downstream Process - Column Purification Protocol
- p1000 micropipette and tips (1/team)
- 1.5 mL microfuge tubes (4/team)
- Microfuge tube rack (1/team)
- Microcentrifuge (1/class)
- p200 micropipette and tips (1/team)
- Binding Buffer (BB) (300uL/team)
- Wash Buffer (WB) (2mL/team)
- Elution buffer (TE) (2mL/team)
- Column Equilibration Buffer (CEB) (3mL/team)
- Chromatography Column + ring stand holder (1/team)
- Small beaker to collect column flowthrough (1/team)
- 10% bleach solution in a beaker - sitting in the sink or inside another container (1/team)
- Downstream Process Batch Record Document

What Students Will Do

- Listen to/watch the Intro to Downstream Process - Protein Purification slide deck
- Follow the Downstream Process - Column Purification Protocol to purify their RFP or GFP
- Each team fills out the appropriate parts of their Downstream Process Batch Record

Teacher Preparation

- Prior to class make copies of
    - Downstream Process - Column Purification Protocol (one per team)
    - Downstream Process Batch Record Document (one per team)
- Prior to class prepare 300uL aliquots of Binding Buffer (BB) (one per team).
- Prior to class prepare 2mL aliquots of Wash Buffer (WB) (one per team).
- Prior to class prepare 2mL aliquots of Elution Buffer (TE) (one per team)
- Prior to class prepare 3mL aliquots of Column Equilibration Buffer (CEB) (one per team)
- Prior to class prepare beakers half full of 10% bleach solution (one per team). Put these in the sink or inside of another container - to contain spills
- Provide each team
    - One p1000 micropipette and tips

- One p200 micropipette and tips
- Four 1.5mL microfuge tubes
- One microfuge tube rack
- Sharpie marker for labeling tubes
- One 300uL aliquot of Binding Buffer (BB)
- One 2mL aliquot of Wash Buffer (WB)
- One 2mL aliquot of Elution buffer (TE)
- One 3mL aliquot of Column Equilibration Buffer (CEB)
- Spray bottle of 70% ethanol
- Paper towels
- Team file folders

Organizer

Time	Activity	Materials
15 minutes	Review the pertinent part of the Intro to Downstream Process - Protein Purification slide deck	Slide deck
5 minutes	Members of all teams put on PPE	Lab coats, gloves, safety goggles
5 minutes	Teams sanitize and prepare their bench space	70% ethanol, paper towels, micropipettes, tips, harvested bacteria
25 minutes	Each team carries out the Downstream Process - Column Purification Protocol.	Column Purification Protocol,1.5mL microfuge tubes, microfuge tube rack, micropipettes, tips, Binding Buffer, Wash Buffer, Elution buffer, Column Equilibration Buffer, Chromatography column + ring stand
5 minutes	Teams label their purified protein tubes and place them in a tube rack to store in the refrigerator. Tubes containing leftover bacterial lysate get stored in the refrigerator also.	Sharpie markers Tube rack
10 minutes	Teams fill out the appropriate portions of their Downstream Process Batch Record Document and file it	Downstream Process Batch Record Document, Team File Folder

Procedure

Introduction to Protein Production in Bacteria

1. Present the Introduction to Downstream Process - Protein Purification slide deck.

Preparation of the bacterial lysate

2. Members of each team put on PPE
3. Each team sanitizes and organizes their bench space
4. Each team follows the Downstream Process - Column Purification Protocol to purify their RFP or GFP from the bacterial lysate.
5. Each team puts their tubes containing the purified RFP or GFP protein in a tube rack to be stored in the refrigerator.
6. Each team fills out the appropriate sections of the Downstream Process Batch Record and files it in their team file.

Downstream Process - Column Purification Protocol

Materials:

1. **Bacterial Lysate:** The tube containing the bacterial solution + lysis buffer that you prepared previously.
2. **p1000 Micropipette tips**: For measuring small volumes of reagents or bacterial cultures
3. **Microfuge tubes of various sizes:** For containing small volumes of reagents or bacterial cultures
4. **Microfuge tube rack**: To hold microfuge tubes.
5. **Liquid waste container**: This is the container will catch all liquids that pass through the column prior to elution of the RFP or GFP.
6. **Sharpie markers**: For correct labeling of samples.
7. **Binding Buffer (BB)**: Provides the salt that enhances binding of RFP or GFP to the column resin
8. **Wash Buffer (WB)**: A lower salt solution that washes off anything that is not specifically bound to the column.
9. **Elution Buffer (TE)**: This is a buffered solution you will resuspend your bacteria in prior to lysis.
10. **Column Equilibration Buffer (CEB)**: This solution prepares the column to be used again.
11. **Downstream Process Batch Record Form**: When properly filled out, this form is a record of your entire upstream process.

Equipment:

1. **Chromatography column + ring stand holder**
2. **p1000 Micropipette**
3. **Small tabletop Microcentrifuge**

Protocol:

Column purification will be carried out by the Downstream Process Technician. The Process Engineer will carry out centrifugation steps.

The Downstream Process Technician and Process Engineer should work with their team members to carry out this procedure.

Dispose of all waste properly. **Do not allow the column to run dry.**

You are STRONGLY ENCOURAGED to check off each step below as you complete it.

Column Preparation

1. Set the liquid waste container under the stopcock. Remove the stopper on the column tip. Remove the lid on the top of the column. See Fig. 1.

2. Carefully open the column by turning the stopcock valve and allow the liquid to begin draining into the waste collection container.

3. Close the valve once there is about 1-2 mm of liquid left above the resin bed.

4. Make sure that no liquid is dripping from the column into the waste container.

Column Purification

5. Spin your lysate tube in the microcentrifuge at 6,000 rpm in a small tabletop microcentrifuge for 5 minutes to pellet cell debris.
   NOTE: Distribute tubes evenly in the microcentrifuge so that their weight is balanced.

6. After spinning, examine the tube. You should see supernatant (liquid) and a solid pellet.

   What color is the supernatant? What color is the pellet? What is contained in the supernatant?

7. Using a p1000 micropipette, carefully remove 200uL of the lysate supernatant without disturbing the cell debris pellet. Dispense the supernatant into a tube labeled Lysate Super.

8. Keep the rest of the spun lysate and store it in the refrigerator.

9. Using a new tip, add 200uL of BB to the Lysate Super tube. Mix gently by pipetting the solution up and down.

10. Add all 400uL of this mixture to the chromatography column. Carefully dispense the solution down the side of the column to avoid disturbing the surface of the resin bed. See Fig. 1 at the end of the protocol to view a photo of a column with all parts labeled.

11. Open the valve and allow the solution in the column to drain into the waste collection container. Close the valve once there is about 1-2 mm of liquid left above the resin bed.

12. Examine the column and locate the red (or green) fluorescent protein. Is it spread throughout the resin bed? Or does it appear to be restricted to a single band?

13. Using a new tip, add 1,000 uL (1 mL) of WB gently down the side of the column.

14. Open the valve and allow the solution in the column to drain into the waste collection container. Close the valve once there is about 1-2 mm of liquid left above the resin.

15. Examine the column and locate the red fluorescent protein. Has the location of the RFP changed in the resin bed?

16. Arrange three 1.5mL microfuge tubes in a microfuge rack. Position one of the tubes under the column stopcock.

17. Using a new tip, add 1,000 uL of TE (Elution Buffer) twice (2 mL total), gently down the side of the chromatography column.

18. With a microfuge tube under the stopcock, open the valve and allow the TE to begin to pass through the column. Does the RFP (or GFP) begin to move?

19. Once you see the RFP get near the bottom of the column, close the valve. Label the next microfuge tube P-RFP, for purified RFP, (or P-GFP for purified GFP) and set it under the stopcock.

20. Open the valve and allow the RFP (or GFP) to drip into this tube. Once the red (or green) has disappeared from the column, close the valve. Measure the volume of your purified RFP (or GFP) and record it on the Downstream Process Batch Record.

21. Cap the P-RFP tube and add your initials to it. It will be stored in the refrigerator until you are ready to determine the protein concentration.

22. Set the waste container back under the stopcock. Open the valve and allow the last of the TE to drip through the column. Close the valve once there is 1-2 mm of liquid above the resin bed.

23. Using a new tip, add 1,000 uL of CEB twice (2 mL total) to the chromatography column to prepare it for reuse.

24. Allow the CEB to drip through the column. Close the valve when there is about 10mm of liquid above the resin bed. Replace the caps on both ends of the column.

25. Pour the contents of the waste collection container down the sink.

26. Store your RFP (or GFP) tube in the refrigerator. Save the rest of your spun bacterial lysate in the refrigerator as well.

27. Fill out the appropriate parts of the Downstream Process Batch Record.

Column with both ends capped. **Column with both ends un-capped.**

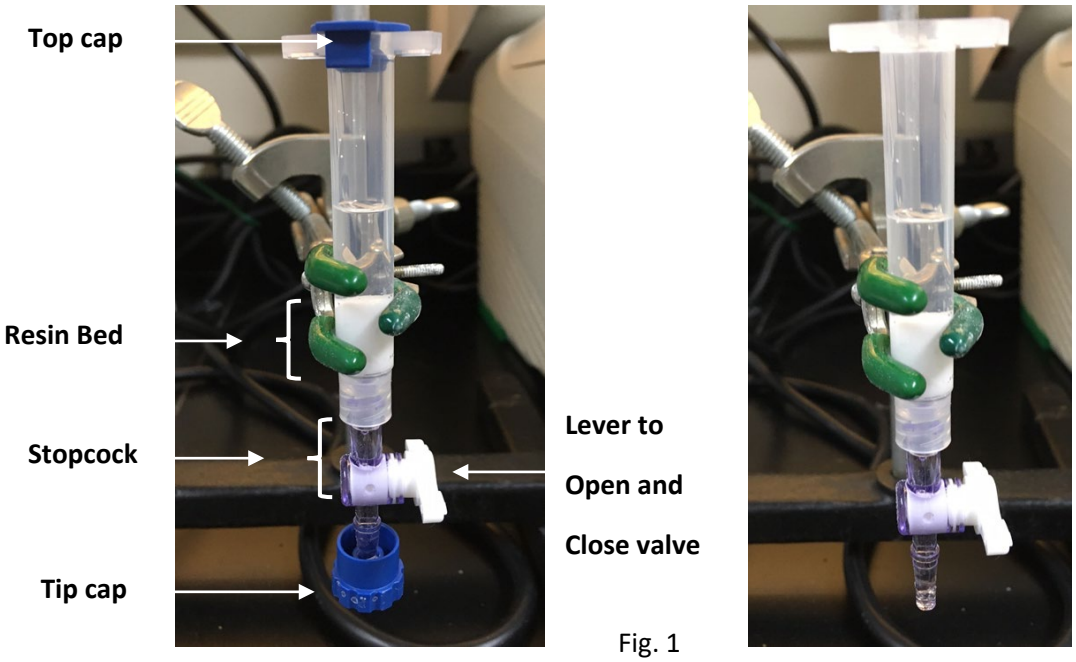


Fig. 1

Biomanufacturing Module 3

Lesson 3 – Protein Concentration Standard Curve using the Bradford Assay

NOTE: It may work well to have teams carry out Lesson 4 while the Process Engineers are carrying out Lesson 3.

Lesson objectives:

Students will understand:

- How to create a protein concentration standard curve using the Bradford Assay.

Essential Question

- How is a standard curve used to determine the protein concentration of a sample of unknown concentration?

Materials:

- Bradford Protein Assay slide deck
- Bradford Assay Standard Curve Protocol
- p1000 micropipette and tips (1/team)
- 1.5 mL microfuge tubes (6)
- 5mL Falcon tubes (7)
- p200 micropipette and tips (1/team)
- Elution buffer (TE) (500ul)
- Bradford reagent (13mL)
- Albumin Standard (100uL)
- Cuvettes (7)
- Container for Bradford Reagent waste (1)
- Protein Concentration Standard Curve Report Document (1/team)

What Students Will Do

- Listen to/watch the Intro to Downstream Process - Protein Purification slide deck
- The Process Engineers from each team will work together to create the protein concentration standard curve
- Follow the Bradford Assay Standard Curve protocol
- Each team fills out the appropriate parts of the Protein Concentration Standard Curve Report

Teacher Preparation

- Prior to class make copies of
    - Bradford Assay Standard Curve Protocol (one per team)
    - Protein Concentration Standard Curve Report Document (one per class)
- Prior to class remove the tube containing the Bradford reagent from the refrigerator and allow it to come to room temperature. Keep it protected from light.
- Prior to class, prepare 13mL aliquots of Bradford Reagent (one per class).
- Prior to class, prepare 100uL aliquots of Albumin protein standard (one per class)
- Prior to class, prepare 400uL aliquots of Elution buffer (TE) (one per class)
- Prior to class, turn on the spectrophotometer to allow it to warm up.

- Prior to class prepare waste containers for the Bradford reagent
- Provide the group of Process Engineers:
    - One p1000 micropipette and tips
    - One p200 micropipette and tips
    - Six 1.5mL microfuge tubes
    - Sharpie marker for labeling tubes
    - One 400uL aliquot of Elution buffer (TE)
    - One 100uL aliquot of Albumin protein standard (2,000 ug/mL)
    - One 13mL aliquot of Bradford Reagent
    - Seven 5mL Falcon tubes
    - Seven cuvettes
    - One microfuge tube rack
    - One test tube rack
    - Spray bottle of 70% ethanol
    - Paper towels
    - Team file folders

Organizer

Time	**Activity**	**Materials**
10 minutes	Present the Bradford Protein Assay slide deck	Slide deck
5 minutes	Members of all teams put on PPE	Lab coats, gloves, safety goggles
5 minutes	Teams sanitize and prepare their bench space	70% ethanol, paper towels, micropipettes, tips, Albumin protein standard, Bradford reagent, microfuge tubes, 5mL falcon tubes, sharpie marker, cuvettes
30 minutes	Process Engineers work together to create the standard curve samples and to collect the standard curve data	Bradford Assay Standard Curve Protocol,1.5mL microfuge tubes, tube rack, micropipettes, tips, 5mL falcon tubes, Albumin protein standard, Elution buffer, Bradford reagent, cuvettes, sharpie marker
10 minutes	Process Engineers share the standard curve data with all teams. Teams fill out the Protein Concentration Standard Curve Report Document and file it	Protein Concentration Standard Curve Report Document, Team File Folder

Procedure

Creation of Protein Concentration Standard Curve Samples and collection of standard curve data.

1. Present the Bradford Protein Assay slide deck.

Preparation of the bacterial lysate

2. Members of each team put on PPE
3. Each team sanitizes and organizes their bench space
4. The Process Engineers from each team work together and follow the Bradford Assay Standard Curve Protocol to create standard curve samples.
5. The Process Engineers read these samples at OD595 on the spectrophotometer, collect the data, and share it with all teams.
6. The Bradford reagent waste is discarded into a ‘Bradford Reagent waste container’. This waste is returned to Shoreline Community College to be properly disposed of.
7. Each team fills out the Protein Concentration Standard Curve Report and files it in their team file.

Bradford Protein Assay Standard Curve Protocol

Materials:

1. **Bradford Reagent (Coomassie Blue):** For determining protein concentration
2. **Micropipette tips**: For measuring small volumes of reagents or bacterial cultures
3. **Microfuge tubes of various sizes:** For containing small volumes of reagents or bacterial cultures
4. **Microfuge tube rack**: To hold microfuge tubes.
5. **Bradford Reagent waste container**: This is the container you will put all Bradford Assay waste into.
6. **Sharpie markers**: For correct labeling of samples.
7. **Albumin protein standard**: A solution of albumin at 2,000 ug/mL that will be used to create a protein concentration standard curve.
8. **Elution Buffer**: The buffer your RFP or GFP was eluted in, which will be used as the blanking solution and also as the diluent for the serial dilution of albumin.
9. **5 mL Falcon Tubes**: For preparing mixing the Bradford reagent and the Albumin protein in
10. **Cuvettes**: For measuring the OD595 of your standard curve samples in the spectrophotometer
11. **Protein Concentration Standard Curve Report**: A document on which to report your standard curve data

Equipment:

1. **Spectrophotometer**
2. **Micropipettes - p1000 and p200**

Protocol:

Done by the Process Engineers. Dispose of all waste properly.

You are STRONGLY ENCOURAGED to check off each step below as you complete it.

1. Remove the tube containing the Bradford reagent from the refrigerator and allow it to come to room temperature.

2. Turn on the spectrophotometer to allow it to warm up.

3. You will need to create a 1:2 serial dilution of the albumin standard (2,000ug/mL).

4. Obtain six 500uL (small) microfuge tubes and label them as follows:

    a. 1,000 ug/mL
    b. 500 ug/mL
    c. 250 ug/mL
    d. 125 ug/mL
    e. 62.5 ug/mL

5. Using a p200 micropipette, put 40ul of Elution Buffer in each microfuge tube. See Fig. 1.

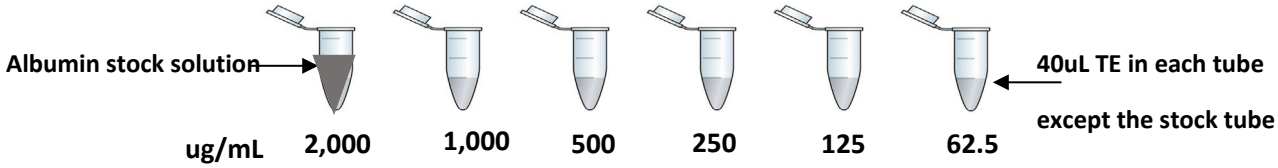


Fig. 1

6. Using a p200 micropipette, remove 40uL of the albumin protein standard (2,000 ug/mL) from the tube it is in. Mix this into the Elution Buffer that is in the tube labeled 1,000 ug/mL. You have now diluted the albumin 2-fold, so it is at 1,000 ug/mL.

7. Continue with the serial dilutions until you have completed the dilution labeled 62.5 ug/mL. See Fig. 2.

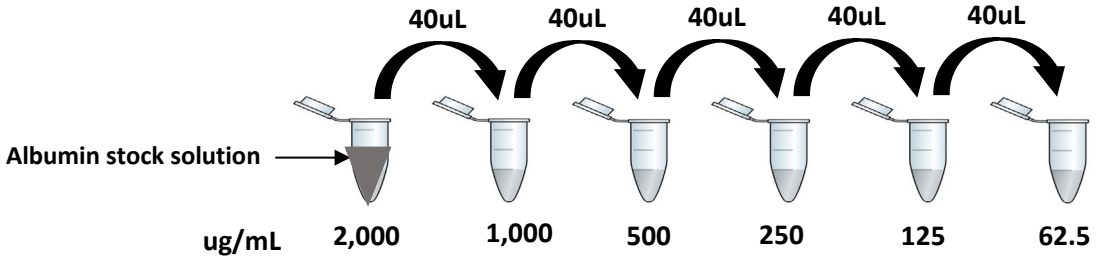


Fig. 2

8. Obtain seven 5mL Falcon tubes. Label them as follows:

    a. 2,000 ug/mL
    b. 1,000 ug/mL
    c. 500 ug/mL
    d. 250 ug/mL
    e. 125 ug/mL
    f. 62.5 ug/mL
    g. 0 ug/mL

9. Remove their caps and set them in a rack. Using a p200 micropipette, put 30uL of the albumin standard in the Falcon tube labeled '2,000'.

10. Using a p200 micropipette, put 30uL of the 1,000 ug/mL solution into the Falcon tube labeled '1,000'. Continue this process until you have put 30uL of each serial dilution solution into the appropriate Falcon tubes.
    NOTE: Be sure to change tips between each sample.

11. Using a p200 micropipette, put 30uL of Elution buffer into the Falcon tube labeled ‘0’. This is the tube contains the ‘blank’ solution.

12. Using a p1000 micropipette, add 1.5mL of Bradford reagent to each tube. Cap each tube tightly and invert twice to mix well. Allow the tubes to sit at room temperature for 10 minutes before reading the samples in the spectrophotometer.

13. Using a p1000 micropipette, transfer the contents of the ‘0’ tube to a clean cuvette.

14. Set the spectrophotometer wavelength to 595 nm.

15. Use this cuvette to ‘blank’ the spectrophotometer. Save this cuvette when you need to blank the spectrophotometer when teams are measuring the concentration of their purified protein.

16. Using a p1000 micropipette, transfer the contents of the ’62.5’ tube to another clean cuvette. Read the absorbance of this solution and record it on the Protein Concentration Standard Curve Report.

17. Remove the solution from the cuvette and put it back in the tube labeled ’62.5’. Use this same cuvette to read the next sample.

18. Repeat steps 16 and 17 for each of the remaining standard curve tubes.

19. Remain ready to read samples for each team.

20. If teams will read their samples during another class period, give the blank tube to the teacher to cover with parafilm to keep for use as a blank.

21. Complete the Protein Concentration Standard Curve Report and make it available to all teams.

22. Each team will use the standard curve data and the Generation of a Standard Curve protocol to plot the standard curve and obtain the equation of the line.

Biomanufacturing Module 3

Lesson 4 – Measuring Protein Concentration using the Bradford Assay

NOTE: It may work well to have teams carry out Lesson 4 while the Process Engineers are carrying out Lesson 3.

Lesson objectives:

Students will understand:

- How to measure the concentration of their purified protein using the Bradford Assay. .

Essential Question

- How is a standard curve used to determine the protein concentration of a sample of unknown concentration?

Materials:

- Bradford Protein Assay slide deck
- Downstream Process - Measuring Purified Protein Concentration Protocol
- p1000 micropipette and tips (1/team)
- 1.5 mL screw cap microfuge tubes (1/team)
- 5mL Falcon tubes (2/team)
- p200 micropipette and tips (1/team)
- Elution buffer (TE) (100ul/team)
- Cuvettes (2/team)
- Bradford Reagent (4mL) (1/team)
- Container for Bradford Reagent waste (1/team)
- Downstream Process Batch Record Document (1/team)
- Heat block (1/class)
- Thermometer (1/class)

What Students Will Do

- Review the Intro to Downstream Process - Protein Purification slide deck if desired
- The Quality Control Technician and the Process Engineer will work together to measure the optical density of the solution of purified RFP or GFP
- Follow the Downstream Process - Measuring Purified Protein Concentration protocol
- Each team fills out the appropriate parts of the Downstream Process Batch Record Document

Teacher Preparation

- Prior to class make copies of
    - Downstream Process - Measuring Purified Protein Concentration Protocol (one per team)
    - Downstream Process Batch Record Document (one per team)
- Prior to class, remove the tube containing the Bradford reagent from the refrigerator and allow it to come to room temperature. Keep it protected from light.
- Prior to class, prepare 3mL aliquots of Bradford Reagent (one per team).
- Prior to class, prepare 100uL aliquots of Elution buffer (TE) (one per team)

- Prior to class, turn on the spectrophotometer to allow it to warm up.
- Prior to class, turn on the heat block and allow it to come to 85 degrees C.
- Prior to class, prepare waste containers for the Bradford reagent
- Provide each team
    - One p1000 micropipette and tips
    - One p200 micropipette and tips
    - One 1.5mL screw-cap microfuge tube
    - Sharpie marker for labeling tubes
    - One 100uL aliquot of Elution buffer (TE)
    - One 3mL aliquot of Bradford Reagent
    - Two 5mL Falcon tubes
    - Two cuvettes
    - One microfuge tube rack
    - One test tube rack
    - Spray bottle of 70% ethanol
    - Paper towels
    - Team file folders

Organizer

Time	**Activity**	**Materials**
10 minutes	Review the Bradford Protein Assay slide deck if desired	Slide deck
5 minutes	Members of all teams put on PPE	Lab coats, gloves, safety goggles
5 minutes	Teams sanitize and prepare their bench space	70% ethanol, paper towels, micropipettes, tips, Bradford reagent, microfuge tubes, 5mL falcon tubes, sharpie marker, cuvettes
25 minutes	The Process Engineer creates a ‘blank’ cuvette and uses it to blank the spectrophotometer. The QC Technician heat denatures the purified RFP or GFP. The QC Technician creates a mixture of the HD purified protein and the Bradford reagent and reads the OD595 of this solution.	Bradford Assay Standard Curve Protocol,1.5mL screw-cap microfuge tubes, tube rack, micropipettes, tips, 5mL falcon tubes, Albumin protein standard, Elution buffer, Bradford reagent, cuvettes, sharpie marker, heat block
10 minutes	Teams fill out the Downstream Process Batch Record Document and file it	Downstream Process Batch Record Document, Team File Folder

Procedure

Creation of Protein Concentration Standard Curve Samples and collection of standard curve data.

1. Review the Bradford Protein Assay slide deck if desired.

Measuring the OD595 of the solution of purified RFP or GFP

2. Members of each team put on PPE
3. Each team sanitizes and organizes their bench space
4. Teams follow the Downstream Process - Measuring Purified Protein Concentration Protocol
    a. The Process Engineer blanks the spectrophotometer.
    b. The QC Technician heat denatures the purified protein.
    c. The QC Technician creates a mixture of the HD purified protein and Bradford reagent and reads this sample at OD595 on the spectrophotometer.
5. The Bradford reagent waste is discarded into a ‘Bradford Reagent waste container’. This waste is returned to Shoreline Community College to be properly disposed of.
6. Each team fills out appropriate section of the Downstream Process Batch Report and files it in their team file.

Downstream Process – Measuring Purified Protein Concentration

Materials:

1. **Bradford Reagent (Coomassie Blue):** For determining protein concentration
1. **Micropipette tips**: For measuring small volumes of reagents or bacterial cultures
2. **Microfuge tubes of various sizes:** For containing small volumes of reagents or bacterial cultures
3. **Microfuge tube rack**: To hold microfuge tubes.
4. **Coomassie waste container**: This is the container you will put all Bradford Assay waste into.
5. **Sharpie markers**: For correct labeling of samples.
6. **Albumin protein standard**: A solution of albumin at 2,000 ug/mL that will be used to create a protein concentration standard curve.
7. **Elution Buffer (TE)**: The buffer your RFP or GFP was eluted in, which will be used as the blanking solution and also as the diluent for the serial dilution of albumin.
8. **Small Falcon Tubes**: For preparing mixing the Bradford reagent and the Albumin protein in
9. **Cuvettes**: For measuring the OD595 of your purified RFP or GFP in the spectrophotometer
10. **Protein Concentration Standard Curve Report**: A document on which the Process Engineers previously reported the standard curve data, the graph of this data and the equation of the best fit line of the data.

Equipment:

1. **Spectrophotometer**
2. **Micropipettes**
3. **Heat Block**

Protocol:

Done by the Quality Control Technician. The Process Engineers will tare (blank) the spectrophotometer.

The QC Technician and Process Engineer should work with their team members to carry out this procedure.

Dispose of all waste properly.

You are STRONGLY ENCOURAGED to check off each step below as you complete it.

1. Remove the tube containing the Bradford reagent from the refrigerator and allow it to come to room temperature.

2. Turn on the spectrophotometer to allow it to warm up. (Process Engineers do this.)

3. Turn on the heat block and set it to 85^{o}C. Use a functional thermometer to verify that the block is actually at 85^{o}C . (Process Engineers do this.)

4. The wavelength of red or green fluorescence interferes with the OD595 readings. For this reason, you will heat denature your purified RFP (or GFP) prior to measuring the protein concentration of the purified protein with the Bradford assay.

5. Using a p200 micropipette, remove 100uL of the purified RFP (or GFP) and put it into a fresh screw cap microfuge tube. Label this tube HD (for heat denatured). A screw cap tube is used so that if pressure builds up in the tube during heating, the lid will not pop off.

6. Place the tube in the 85^{o}C heat block for 5 minutes. When you pull it out the purified protein should be clear rather than pinkish (RFP) or greenish (GFP). Why do you think the color has changed?

7. Obtain a small Falcon tubes. Label it HD RFP (or GFP).

8. Remove the caps and set it in a rack. Using a p200 micropipette, put 30uL of the heat denatured protein in the Falcon tube.

9. Using a p1000 micropipette, add 1.5mL of Bradford reagent to the tube. Cap the tube tightly and invert twice to mix well. Allow the tube to sit at room temperature for 10 minutes before reading the sample in the spectrophotometer.

10. Set the spectrophotometer wavelength to 595 nm.

11. Use the blank cuvette from the preparation of the albumin standard curve to 'blank' the spectrophotometer. If you do not still have the blank from the previous collection of standard curve data, make a new one.
    a. Using a p200 micropipette, put 30uL of Elution buffer (TE) into a Falcon tube. Using a p1000 micropipette, add 1.5mL of Bradford reagent to the tube. Cap the tube tightly and invert twice to mix well. Allow the tube to sit at room temp. for 10 minutes before transferring the sample to a cuvette and using it to blank the spectrophotometer.

12. Using a p1000 micropipette, transfer the contents of the HD tube to a clean cuvette. Read the absorbance of this solution and record it on the Downstream Process Batch Record.

13. Remove the cuvette from the spectrophotometer. Dump the Coomassie solution into the Coomassie waste container. Dispose of the cuvette in biohazard waste.

14. Use the OD595 reading from your sample and the equation of the line from the albumin standard curve assignment to calculate the concentration of your purified RFP (or GFP). Record this value on the Downstream Process Batch Record.

15. Using the total volume of your purified RFP sample (eluate) recorded previously and the protein concentration of your sample, calculate your total protein yield. Record this value on the Downstream Process Batch Record.

16. Complete the Downstream Process Batch Record and file it in your Team file.

Biomanufacturing Module 3

Lesson 5 – Graphing a protein concentration standard curve in Excel

Lesson objectives:

Students will understand:

- How to graph a standard curve.

Essential Question

- How is a standard curve used to determine the protein concentration of a sample of unknown concentration?

Materials:

- Downstream Process - Measuring Purified Protein Concentration Protocol
- Computer or Tablet that can run Excel
- Standard curve data from Module 3, Lesson 3 - found in the Protein Concentration Standard Curve Report Document
- Downstream Process Batch Record Document (1/team)

What Students Will Do

- Team members will each use Excel to graph the protein concentration standard curve data generated in Module 3, Lesson 3
- Follow the Graphing a Protein Concentration Standard Curve in Excel protocol
- Each team fills out the appropriate parts of the Downstream Process Batch Record Document

Teacher Preparation

- Prior to class make copies of
    - Graphing a Protein Concentration Standard Curve in Excel Protocol (one per team)
    - A copy of the Standard Curve Data (Module 3, Lesson 3)
    - Downstream Process Batch Record Document (one per team)
- Provide each team
    - A computer or tablet that can run Excel

Organizer

Time	Activity	Materials
10 minutes	Brief introduction to Excel or Google Sheets	
20 minutes	Team members create a graph of the standard curve data generated in Module 3, Lesson 3	Graphing a Protein Standard Curve Protocol, Protein Concentration Standard Curve Report Document

10 minutes	Teams use the equation of the line from the standard curve and the OD595 reading of their purified protein sample to calculate the concentration of their purified protein.	Graphing a Protein Standard Curve Protocol, Downstream Process Batch Record Document
10 minutes	Teams fill out the Downstream Process Batch Record Document and file it	Downstream Process Batch Record Document, Team File Folder

Procedure

Graphing of Protein Concentration Standard Curve Sample Data and calculation of the concentration of the purified protein sample.

1. Introduce the Excel or Google Sheets graphing program.

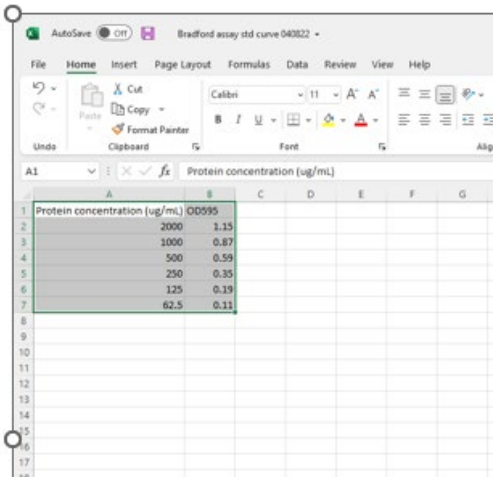
Graphing the standard curve data and calculation of the concentration of the purified protein.

2. Members of each team follow the Graphing a Protein Standard Curve Protocol to create a standard curve and get the equation of the best fit line.
3. Each team uses the equation of the line and their purified protein sample OD595 reading to calculate the concentration of their purified protein sample.
4. Each team fills out the appropriate section of the Downstream Process Batch Report and files it in their team file.

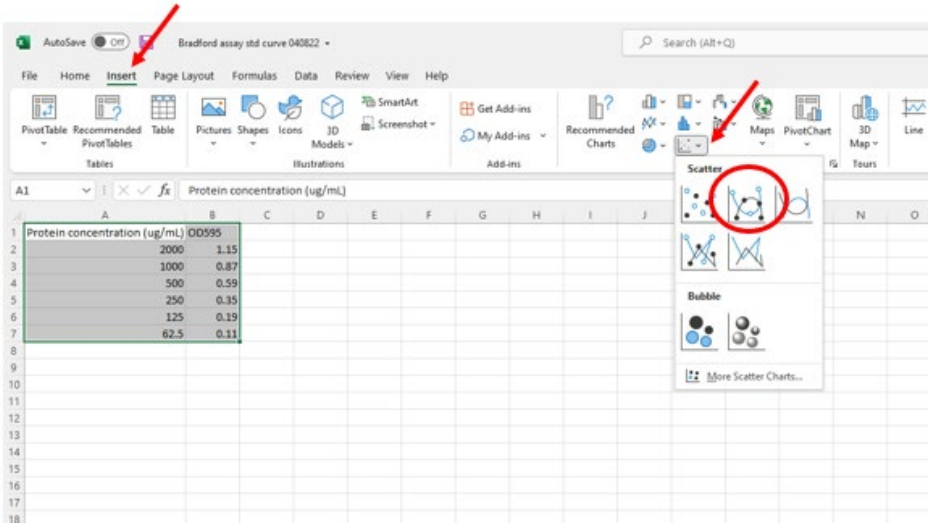
Graphing a Protein Standard Curve in Excel (PC)

Each team will create a protein concentration standard curve using the Excel program, a commonly used spreadsheet program. Each team will use the equation of the standard curve line and the OD595 reading of their purified protein to calculate the concentration of their purified protein.

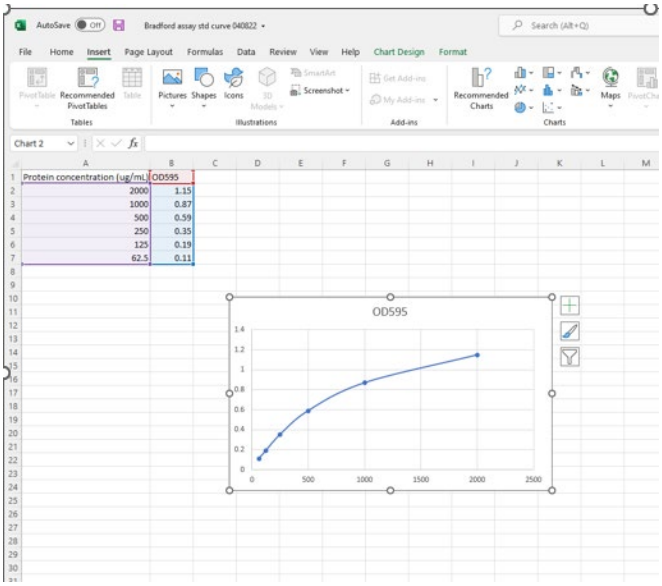
1. Open an Excel spreadsheet.
2. Create a heading in box A1 called 'Protein Concentration (ug/mL)'. Create a heading in Box B1 called 'OD595'. In put your data from highest concentration to the lowest as shown in the example below.



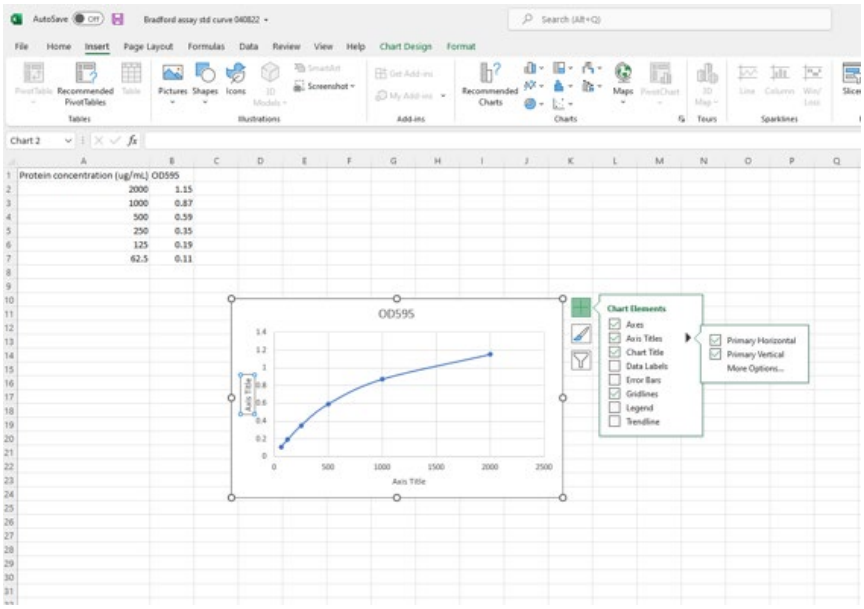
3. Select the entries in both column A and B. Select the 'Insert' tab at the top of the page (indicated by the slanted red arrow). Then select the line graph icon (indicated by the horizontal red arrow). When that window opens, select the type of graph shown in the figure below (red circle).



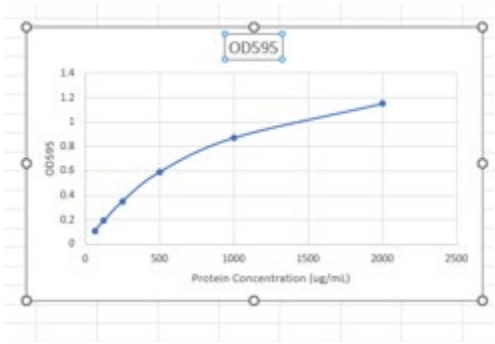
4. This will plot your data with the OD595 readings on the y-axis and the protein concentration on the x-axis. The axes are not properly labeled yet and the graph title is incorrect.



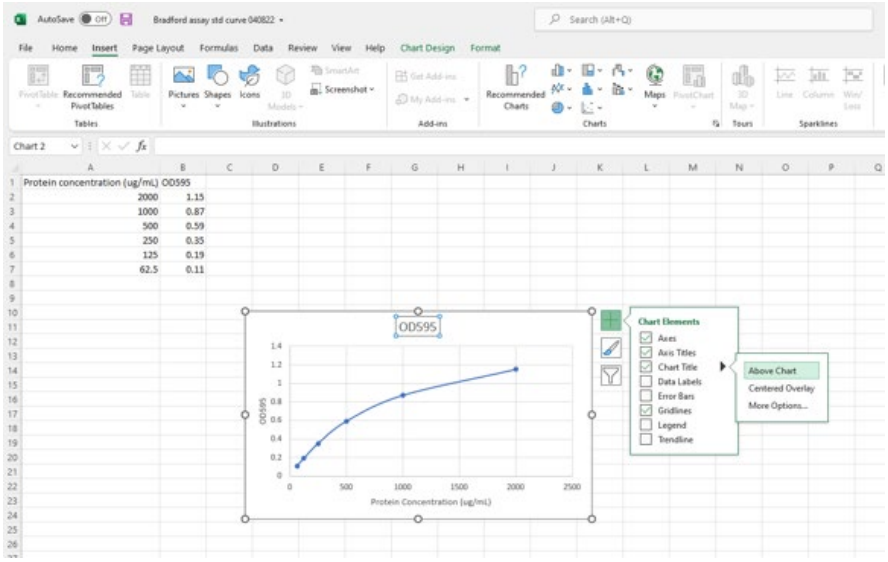
5. Click inside of the graph box to make the 3 icons appear to the right of the graph box. Click on the '+' icon. Make sure the 'Axis Titles' box is checked. Click on the arrow next to 'Axis Titles' and 'Primary Horizontal' and 'Primary Vertical' choices will appear. Make sure both of those boxes are checked. Now the axis labels will be activated and you can click on them and rename the axes.



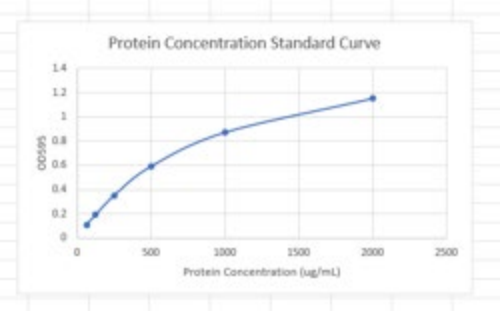
6. Change the x-axis label to: Protein Concentration (ug/mL). Change the y-axis label to: OD595. Now the graph should look like this.



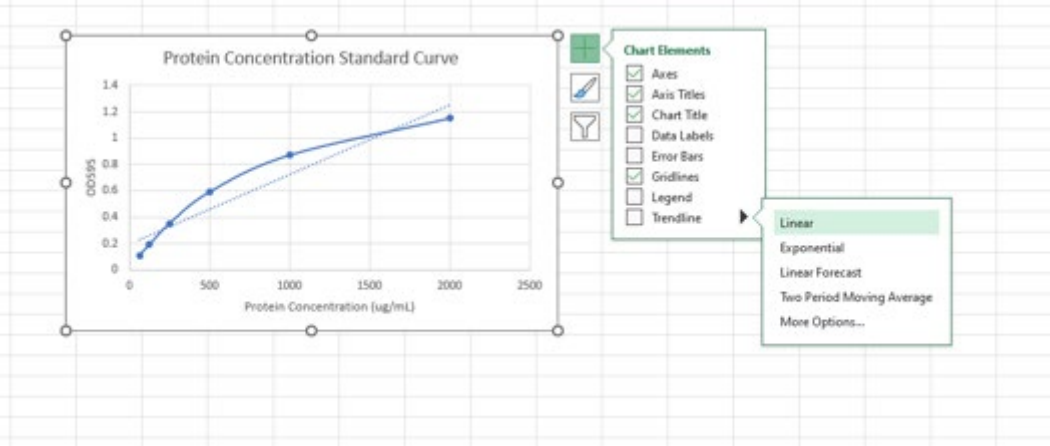
7. The chart title will still be incorrect. Click on the '+' icon again. Make sure the 'Chart Title' box is checked. Click on the arrow to the right of the 'Chart Title' box. This will open a window with choices for where the title appears. It also activates the chart title text. Click on the chart title text and change it to: Protein Concentration Standard Curve.



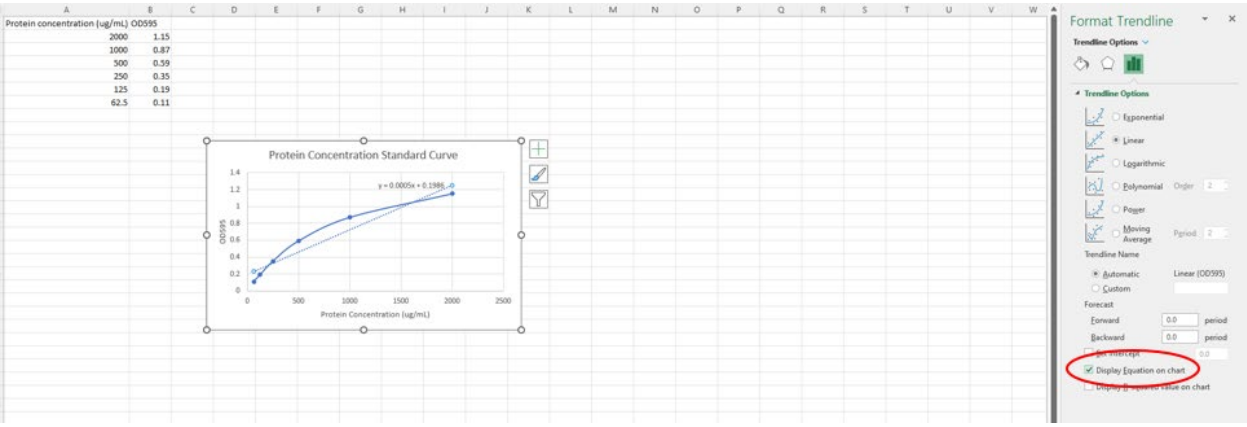
8. Now your graph should look like this.



9. Now you need to plot a linear 'best fit' line. Click inside the graph box to get the '+' box again. Click the 'Trendline' box and choose 'Linear'. Now a linear best fit line will display.



10. To find the equation of the line, click on the '+" button again. Go to 'Trendline'. This time choose 'More Options'. A menu will appear on the right side of the screen. Click on the 'Display equation on chart' box. Now the equation of the 'best fit' line will display in the graph box.



11. You can use this equation to calculate the concentration of your unknown – your purified protein. The equation is written in the form of: **y = mx + b.**
m is the slope of the line.

b is the y-intercept of the line.
y is the OD595 reading.
x is the concentration of the sample.

To solve for x use: $$x = \frac{y-b}{m}$$

12. So, in the case of the example line, the equation is:

    y = 0.0005x + 0.1986

13. If a sample of unknown concentration (like the purified protein) gave an OD595 reading of 0.65, we can calculate its concentration. In this case:
    y = 0.65

    m = 0.0005

    b = 0.1986

    SO : $$x = \frac{0.65- 0.1986}{0.0005} = 902.8 \text{ ug/mL}$$

14. Once you know the protein concentration of your purified protein, you can calculate your protein yield by multiplying the concentration by the volume of the sample you collected. Make sure your volume units are the same when you do the calculation.

15. Calculate the protein concentration and yield of the protein that you purified. Enter this information in the Downstream Process Batch Record.

Biomanufacturing Module 3

Lesson 6 – Creating a product label for a vial of purified RFP

Lesson objectives:

Students will understand:

- What information should be displayed on a product label.

Essential Question

- Why should vials of medicines/therapeutics have product labels?

Materials:

- Creating a Vial Label Assignment
- Paper
- Pencils
- Colored pens or pencils

What Students Will Do

- Listen to/watch the Product Labeling slide deck
- Each team member will create a label for a vial of their purified RFP (or GFP)

Teacher Preparation

- Provide each team:
    - Scratch paper
    - Pencils
    - Colored pens or pencils
    - Creating a Vial Label Assignment

Organizer

Time	Activity	Materials
10 minutes	Present the Product Labeling slide deck	Slide deck
40 minutes	Each team member creates a vial label for their purified RFP or GFP product	Paper, Pencils, Colored pens or pencils

Procedure

Creation of Product Labels

1. Present the Product Labeling slide deck.
2. Team members create their labels.

Creating a Product Label

1. Each team member will create a product label for their purified RFP.
   Your label will not contain all of the information required by the FDA. But hopefully this assignment will get you thinking about what type of information should be on medical product labels.

2. Your label should be eye-catching but also easy to read.

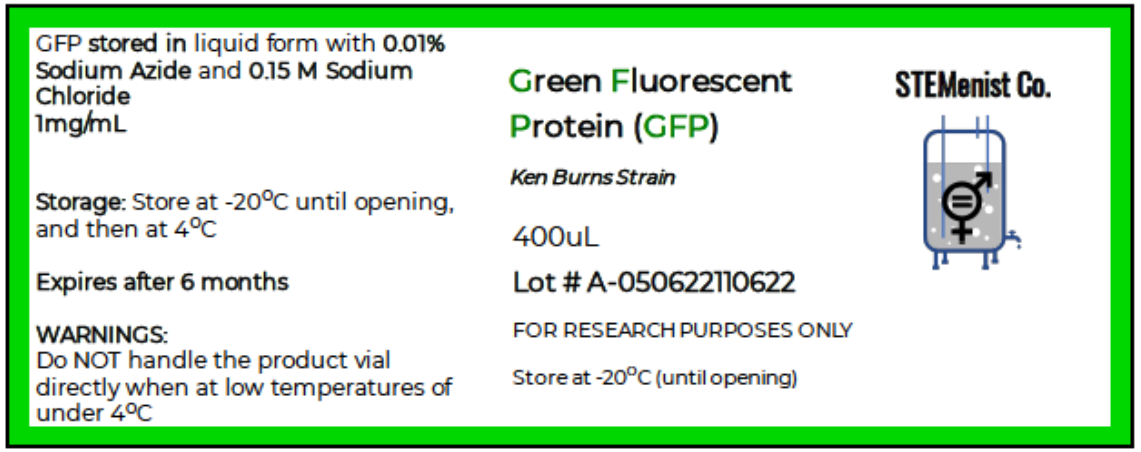
3. You may use color and images.

   For this assignment please feel free to use more color and images than you see on most of the label examples in the Creating a Product slide deck. Be creative!

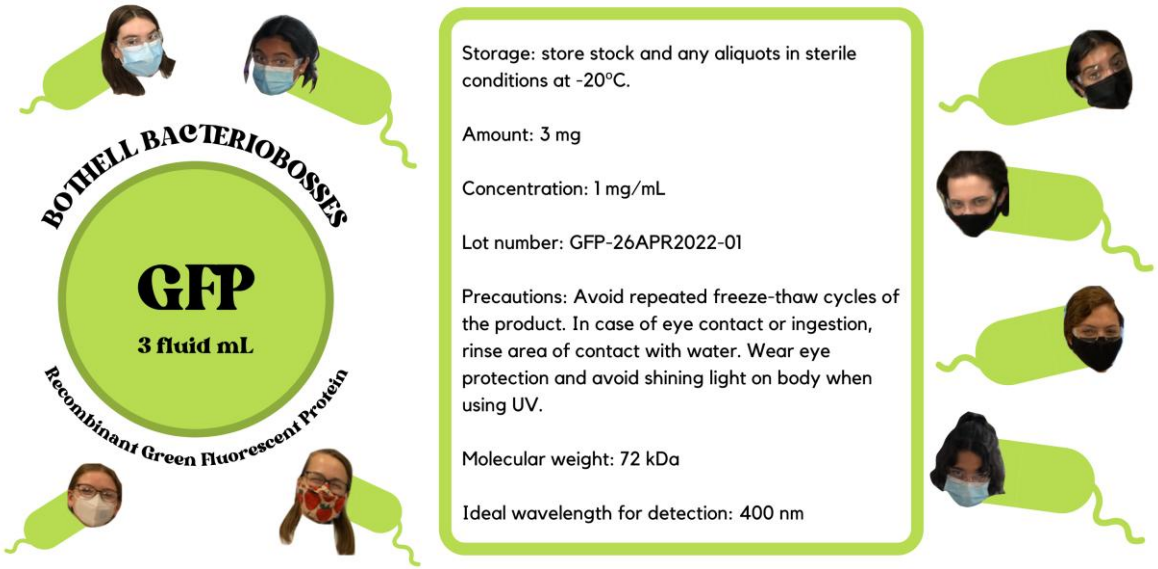
4. Your label should contain the following information:
   - Product Name – Each team should create a name for their purified RFP (or GFP) product
   - Produced by: Team Name
   - Total Volume of purified protein:
   - Purified protein Concentration:
   - Storage: Store at 4 ^{o}C
   - Lot # - The Lot # should be RFP-Purification date-Team Name Abbreviation (not longer than 6 characters)
     Example: RFP-08242022-PPP
   - Warning: For Research Purposes Only
   - Team Logo

5. Print or draw your product label out in a size of approximately 4 inches x 5 inches

Example Student Product Label Designs from BioPath 2021-2022







Team Logo

Downstream Process Batch Record

Team Name:_____

1. Harvest of bacterial cells

Total Bacteria Harvested (mL)	Name	Initials	Date Month Day Year Ex: 03242022

2. Preparation of a bacterial lysate:

Amount of Supernatant Recovered (mL)	Name	Initials	Date Month Day Year Ex: 03242022

3. Column chromatography:

Task	Name	Initials	Date Month Day Year Ex: 03242022
Column Preparation			
Column Loaded			
Column Washed			
RFP or GFP Eluted			
Volume of Eluate _____ mL			
Heat Denaturation			
Coomassie Assay/ Spectrophotometer Preparation			
OD_{595}			
Concentration of Purified Protein			

_____ mg/mL			
Purified Protein Yield **_____ mg**			
	Name	**Initials**	**Date** **Month Day Year** **Ex: 03242022**
QC Technician:			
QA Technician:			

Protein Concentration Standard Curve Report

Albumin (ug/mL)	OD_{595}
0 (TE)	
62.5	
125	
250	
500	
1000	
2000	

Standard Curve Plot (OD_{595} vs mg/mL). Please copy/paste below

Equation of a Line: $y=mx + b$

Solve for x to find concentration in mg/mL:

Names of Process Engineers	Team Name	Initials	Date Month Day Year Ex: 03242022

Extension Activities for Module 3

Virtual Tour of Downstream Processing

Purpose:

This activity is designed to help you familiarize yourself with the overall processes and equipment used in downstream processing. By completing this assignment, you will be able to:

1) Describe the purpose of each step during the capture and purification of a monoclonal antibody
2) Put together the things you've learned about the major steps in downstream processing

Instructions:

1. Navigate to the Downstream Processing: eLearning Modules website.
   If the link above does not work, try this one:
   https://wwws.mc3.edu/downstreamprocessing/story_html5.html
2. Click on **Module 2: Downstream Process**, which is an interactive that will introduce you to downstream processing.
   - Note: be aware the voice is quite robotic ☺
3. Use the questions below to guide you through the interactive.
4. Answer the associated questions while going through the interactive, typing directly into this document.
5. Once you are finished with the questions on this sheet, go back to the Canvas assignment and submit your completed assignment.

Note: the website will allow you to save your spot if you want to come back to it later.

Assignment Questions:

1. Warm-up question: This simulation describes the purification of monoclonal antibodies. What is a monoclonal antibody?

2. At the top of your screen you should see "**Glossary**" in the toolbar. Use this glossary to review the following terms. In your own words, briefly describe what each term means.
   a. API
   b. mAb
   c. Clarification
   d. Filtrate
   e. Retentate
   f. Continuous centrifuge
   g. CHO
   h. Capture chromatography
   i. Intermediate/Polishing Chromatography
   j. Ligand

3. Click on the "Bioreactor" at the beginning of the downstream process and proceed through the explanation. (Once you are finished with the Bioreactor segment, it will bring you back to the homepage and show a check mark next to the Bioreactor step.)
    a. This step should be a review of what you have learned already. What was one thing that stood out to you?

4. Click on the next step, Continuous Centrifuge, and proceed through the explanation.
    a. What is the purpose of centrifugation during this processing stage?

    b. How does centrifugation work?

    c. What is being separated?

5. Click on the next step, Depth Filtration, and proceed through the explanation.
    a. What is the purpose of depth filtration?

    b. How does it work?

    c. What is removed at this stage?

6. Click on the next step, 0.22 μM Sterile Membrane Filtration, and proceed through the explanation.
    a. What is the purpose of this step?

    b. What does "0.22 μM" refer to?

        i. Why is this size important?

    c. What type of filtration method is used here?

        i. Describe how this filtration method works.

    d. What is removed at this stage?

7. Click on the next step, Hold Vessel (10,000 L), and proceed through the explanation.
    a. What is the purpose of this step?

    b. Why is it important that the vessel is held under controlled conditions?

    c. What things might be in the vessel at this stage? Try to include everything you can think of.

    d. Why is it important to clarify the mAb before putting into the hold vessel? Why wouldn't you put the contents of the bioreactor directly into the hold vessel?

8. What is the overall purpose of the clarification stage?

    a. At the end of the recovery stage, what is the material left that you will continue to process? In other words, what is the material that moves on to purification?

9. Now we will proceed with the purification steps. Click on the Protein-A Capture Chromatography step.
    a. What is the primary goal of this step?

    b. How does this type of chromatography work?

    c. What is retained on the column?

    d. What is the purpose of the following steps:
        i. Equilibration
        ii. Load
        iii. Wash
        iv. Elution

10. Next click on the Viral-inactivation/Low pH Hold Vessel (2000L).
    a. What is the purpose of this step?

    b. What factors impact the effectiveness of viral inactivation?

11. Next click on the 0.22μm Sterile Membrane Filtration step.
    a. What is the purpose of this step?

    b. What is removed during this step?

12. Move to Cation-Exchange Chromatography.
    a. What is the purpose of this step?

    b. What is a non-native isoform?

    c. How does IEC work?

    d. Why is pH important at this step?

    e. What is salt used for during this step?

13. Next click on the Flow Through Hold Vessel (500L)
    a. What is the purpose of this step?

14. Move to Anion-Exchange Chromatography.
    a. What is the purpose of this step?

    b. What binds to the column?

    c. What is in the flow-through?

    d. How is this step different than Cation-exchange chromatography?

15. Next click on the 0.1μm Viral Clearance Nanofiltration step.
    a. What is the purpose of this step?

    b. What type of filtration flow is used?

16. Now move to Ultrafiltration/Diafiltration. I know these are new terms for you, so before you move through this step, please check out this short intro to the terms.
    a. What is the two-fold purpose of UF/DF?

    b. Why is high concentration necessary?

    c. Why is a formulation buffer necessary?

    d. What type of filtration flow is used at this step?

    e. Does the antibody pass through the filtration membrane? Why or why not?

17. Finally click on the Bulk Fill step.
    a. What is the purpose of 0.2μm filtration before bulk fill?

    b. What needs to happen before the drug product is ready for shipment?