

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.