

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.