

## **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.