TEACHER GUIDE
Making Medicine

LEARNING UNDEFEATED
Educate | Elevate | Empower

Biomanufacturing for Health

Grade level(s): 10th – 12th grades

Time: 45-60 minutes

Next Generation Science Standards: HS-PS1-3, HS-PS4-1,

TEKS: CHEM.7D, BIOL.7D, PHYS.8F

Virginia Science Standards of Learning: PS.3, PS.7

ACTIVITY OVERVIEW

Students will perform protein purification using column chromatography to gain a greater understanding of the biomanufacturing process of going from a cell to a protein to a product. Students will separate a mixture based on the chemical properties of proteins using ion exchange chromatography and then will use spectroscopy to test the purity of their product. The goal of the activity is for students to learn about the multiple steps to the biomanufacturing process and how all the different fields of science and engineering come together when we apply it to make medications.

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ALIGNMENT TO STANDARDS

NGSS:

HS-PS1-3. Plan and conduct an investigation to gather evidence to compare the structure of substances at the bulk scale to infer the strength of electrical forces between particles

HS-PS4-1. Use mathematical representations to support a claim regarding relationships among the frequency, wavelength, and speed of waves traveling in various media.

TEKS:

CHEM.7D- analyze the properties of ionic, covalent, and metallic substances in terms of intramolecular and intermolecular forces.

BIOL.7.D- discuss the importance of molecular technologies such as polymerase chain reaction (PCR), gel electrophoresis, and genetic engineering that are applicable in current research and engineering practices

PHYS.8F -investigate the emission spectra produced by various atoms and explain the relationship to the electromagnetic spectrum

Virginia Science Standards of Learning:

PS.3A The student will investigate and understand that matter has properties and is conserved in chemical and physical processes. Key ideas include a) pure substances can be identified based on their chemical and physical properties.

PS.7 - Ps.7 The student will investigate and understand that electromagnetic radiation has characteristics. Key ideas include a) electromagnetic radiation, including visible light, has wave characteristics and behavior; and b) regions of the electromagnetic spectrum have specific characteristics and uses

LEARNING OUTCOMES

Students will know:

- The cellular process of making proteins can be used in biomanufacturing to create products used in the medical field
- Proteins can be identified by both their physical and chemical properties

Students will understand:

- Molecules like proteins can be separated by their different chemical properties
- Changing the strength of a charge on a protein can change its ability to form ionic bonds
- How different molecules have different absorption and excitation properties

Students will be able to:

- Use a column to separate proteins based on physical properties
- Use a spectrophotometer

CAREER CONNECTIONS

Cell Culture Technician

Cell culture technicians grow and maintain various types of cells in large flasks or bioreactors. They are expected to be familiar with different cell culture techniques, media preparation, cell isolation, and molecular biology. They are the ones who ensure that cells that produce a product a healthy and growing within federal regulations. An associate or bachelor's degree in biotechnology or related field is usually required with a certification in cell culture.

Biomanufacturing Technician- Downstream

The downstream Biomanufacturing Technician are the ones responsible for harvesting, testing, purifying, and packaging the products. The technician's goal is to isolate a pure product that is at a high yield in the most efficient manner. They monitor devices to ensure and perform purification steps such as column chromatography, ultrafiltration, and other methods to purify a product. An associate's degree in applied science such as biotechnology or bioprocess technology is required with the possibility of an advanced position with a bachelor's degree.

Quality Assurance Specialist

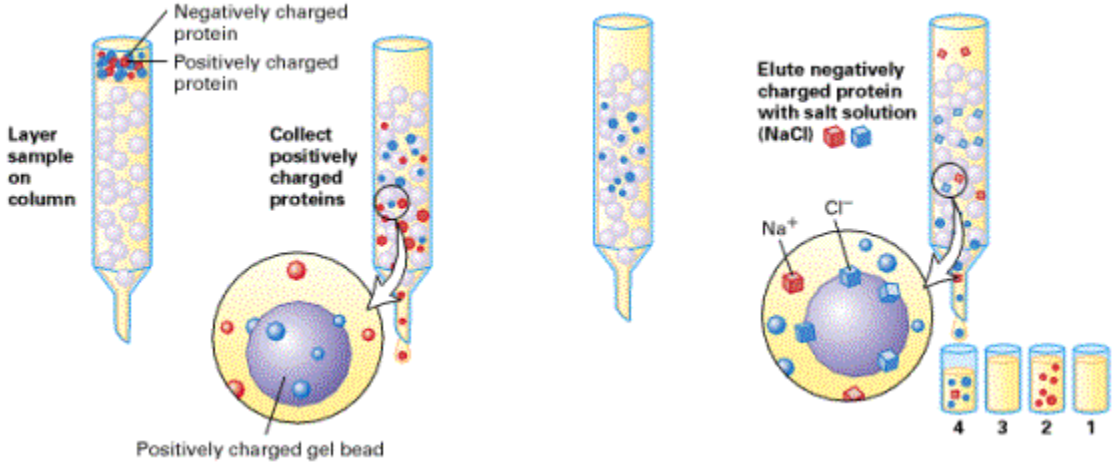
The Quality Assurance Specialist oversees the biomanufacturing system and ensures that staff are maintaining proper practices and procedures to help reduce the chance of a mistake and solve problems as they appear. They are direct supervisors to Quality Control technicians and are responsible for checking that equipment are installed correctly and working properly, inspecting materials for high quality, and documenting and signing off on all steps of the biomanufacturing process. A bachelor's degree in biology or related field is usually required.

Information taken from https://biotech-careers.org/. More resources on biotechnology, biomanufacturing, and related careers can be found on their website.

BACKGROUND INFORMATION

Chromatography describes the different techniques used to separate different types of mixtures into their different components. There are different ways of separating component in a mixture differs based on the chemical and physical properties of the mixtures. Column chromatography is the most common method in biotechnology of separating a mixture of biomolecules such as proteins. There are four major types of column chromatography- Affinity, Hydrophoic Interaction, Ion Exchange, and Size Exclusion. Each has its advantages and disadvantages depending on the end goal.

Ion exchange chromatography works by separating molecules by their charge. The matrix is composed of beads that are either positively charged (anion) or negatively charged (cation). When the mixture is added to the column, the molecules that have the opposite charge of the matrix will form ionic bonds and stick to the beads. All other molecules will pass through the column. Proteins and other molecules can then be eluted by adding a counter-charged buffer of varying strengths. Molecules that are bound weakly will be eluted with a weak buffer and molecules with a stronger bond require a stronger buffer to be eluted. This allows one to elute different molecules at different times by changing buffer strength.



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PRE-LABORATORY ENGAGEMENT

- Introduce students to biomanufacturing by watching this short video from the National Institute of Innovation in Manufacturing Biopharmaceuticals: VIDEO
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POST-LABORATORY ENGAGEMENT

- Have students watch this video on synthetic biology from PBS: LINK
- Open the class to different discussions on various topics. Students should be able to research in groups on the different aspects of synthetic biology from a health perspective as well as social perspective. Examples:
    - Have students discuss the advantages of synthetic biology and how we can use DNA tools to engineer novel proteins
    - Bioengineering is using biology to solve problems. What other global problems can we use synthetic biology to solve?
    - Is synthetic biology creating life? What problems do you see with synthetic biology?

Preparations/ Activity Set Up

MATERIALS

- Edvotek Ion Exchange Chromatography Kit #243
- Distilled Water
- 14ml Test tubes
- Ring stand with clamps
- 1.5ml microcentrifuge tubes- clear
- Transfer pipettes
- P1000 micropipettes with tips
- Waste cups
- Spectrophotometers
- 1.5ml Semimicro cuvettes

Buffer Preparation

0.5M Potassium Acetate (KOAc)

1. Dilute the concentrated KOAc, pH 6.0 by adding 25ml of concentrate to 100ml dH_2O
2. Aliquot 10ml into 10 conical tubes or small containers. Label 0.5M KOAc.

0.01M Potassium Acetate

1. Add 15ml of 0.5M KOAc to 735ml of dH_2O.
2. Aliquot 30ml into 50ml conicals and label 0.01MKOAc

* If making your own KOAc concentrate, be sure to pH to 6.0 before creating dilutions.

Ion Exchange Matrix

It is essential that there are no clumps in your matrix. Therefore, it is recommended that you use a stir plate to mix.

1. Add your CM-cellulose ion exchanger to a 250ml glass beaker
2. Add 150ml of 0.01M KOAc to the beaker. Mix with stir bar until all clumps are removed. You may need to use a clean spoon or spatula to break large clumps.
3. Shut off the stir plate and let the exchanger settle for at least 5 minutes
4. Discard most of the liquid.
5. Add 150ml of FRESH 0.01M KOAc to the exchanger. Mix with stir bar until no clumps. Let settle.
6. Discard most of the liquid
7. Add 50ml of FRESH 0.01M KOAc to the exchanger. Mix with stir bar until fully dispersed.
8. Pour out 6ml aliquots in 14ml conical tubes. Be sure to swirl the matrix in between each pour to ensure it is mixed.

Sample Mixtures and Standards

Protein “Samples”

1. Add 2ml of Blue/Green Dye to bottle of red dye. Mix well by inversion.
2. Create 0.5ml aliquots labelled “Sample”

Standard Samples

1. Create 1ml aliquots of each dilution following the table below. Adjust volumes for estimated number of student groups.
2. Stock solution is Blue/Green Dye

Label	DILUTION	
1 mg/mL	STOCK	
0.5 mg/mL	16ml of 1mg/mL	16mL of H_2O
0.25mg/mL	16ml of 0.5mg/mL	16mL of H_2O
0.125mg/mL	16ml of 0.25mg/mL	16mL of H_2O
0.0625 mg/mL	8ml of 0.125mg/mL	8mL of H_2O

Lab Setup

Station set for groups of 2 or 3:

- Ring stand with column clamped
- 30ml of 0.01M KOAc

- 10ml of 0.5M KOAc
- 0.5ml “Sample”
- 6ml of ion matrix slurry
- Small waste cup
- 3 transfer pipettes
- p1000 micropipette with tips
- 12 empty 1.5ml Microcentrifuge tubes
- 12 disposable cuvettes

For table:

- Vernier Spectrophotometer with Vernier Spectral Analysis

Quantification Extension

- Standard Curve Samples
- 6 disposable cuvettes

Lesson Plan

Introduction

- Begin the class by telling students we are going to be working in a biomanufacturing facility to assist in developing a new medicine to treat heart disease
  - Ask students to think about what are the different types of medicine and how they are made?
    - Can discuss by raising hands or having discussion in groups for 2 minutes and sharing
    - Examples:
      - Chemical analogues- Using chemistry to create substances that mimic biology Ex: Plavix, Ibuprofen
      - Biologics- Produced using living systems. Ex: Insulin, Penicillin,

- Explain that biomanufacturing is the process of making products from living systems.
  - Can be a natural product or genetically engineered
  - Small molecules like RNA and proteins are most common
- Ask what biomolecule contains the “instructions” to make proteins and RNA?
  - DNA contains the “code” to make these molecules
  - By using the natural system of cell replication and protein synthesis, biomanufacturing uses cells as the factory to create biologics that can be used for medicines, vaccines, and by manipulating the DNA sequence- things like biofuels, fragrances, extracts, etc.
- Explain that today we are going to be taking part in the downstream part of biomanufacturing.
  - Upstream Processing- Developing and growing the cells

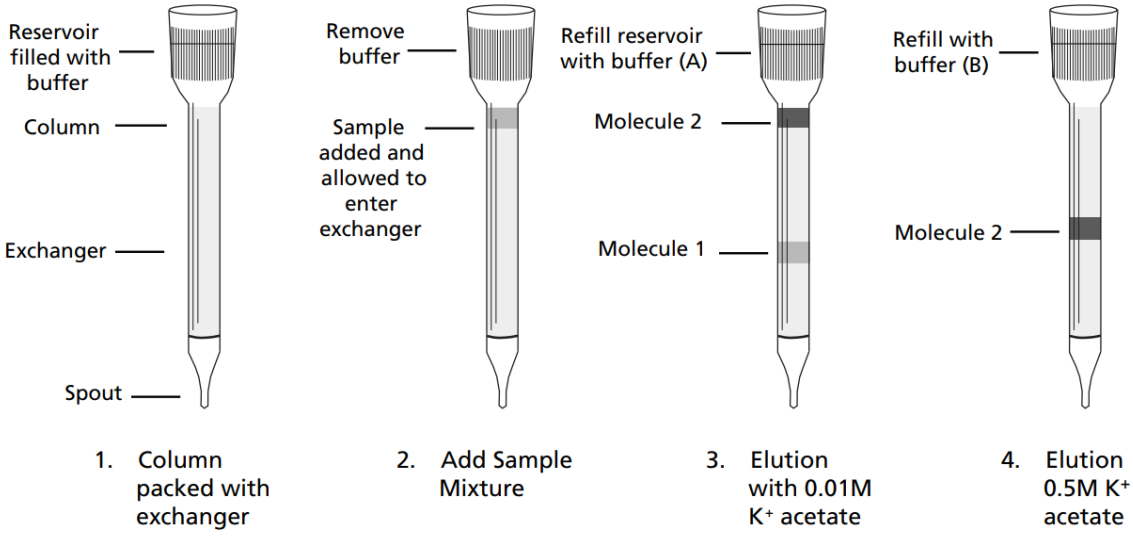
- Downstream Processing- Isolating the product from the cells and ensuring that it meets regulations for purity, efficiency, and yield

Experiment Setup

❖ Introduce to students the problem:

The upstream team at the bioprocessing facility has sent over a cell extract that contains our protein of interest. However, it seems to be contaminated with a protein with a slightly different charge and color. To separate our proteins by charge, we will use a process called ion exchange chromatography. Ion exchange chromatography works by using buffers of different ionic strength or salt to separate molecules by their charge. Molecules will then elute off the column when the buffer is strong enough to weaken the ionic bond between the molecule and the column matrix.

OVERVIEW OF PROCEDURE



- ➢ Demonstrate how to set up a column on the ring stand with waste cup below it.
- ➢ Review the materials in front of the students:
  - Transfer pipette
  - Cellulose Matrix (negative charge)
  - 0.01M Potassium Acetate (low salt buffer)
  - 0.5M Potassium Acetate (high salt buffer)
- ➢ Packing the column
  - Use the transfer pipette to wash the column by filling it to the reservoir
    - This is needed to equilibrate and wash the column
  - Demonstrate how to remove cap and let liquid flow into waste cup and then recap- allow students to follow

- Demonstrate how to add the matrix to column
  - Add slowly
  - Avoid clumps and bubbles
  - Approximately 5ml
  - Top off with 0.01M KOAc
  - Let the excess volume flow through- but not dry out before closing
  - Remove excess liquid from top
- Have students fill their columns- being sure they are well packed

➢ Explain to the students that the column matrix is negatively charged and the proteins in our cellular mixture are positively charged
- What will happen when we add our mixture to the column?
  - Proteins should bind to matrix
- Have students work as tables to answer the question of which proteins will elute with different buffers and make a hypothesis
  - Discuss after how the weak buffer will disrupt the ionic bond of our contaminant protein but not our protein of choice as it has a stronger positive charge, and therefore is bound more strongly.
  - Salts can change the charge of our reactants, making them "less positive" and weakening the ionic bonds. The stronger the salt, the better it is as disrupting the bond

Protein Purification

❖ Review how to use a micropipette
- How to pick up a liquid and how to dispense a liquid
- Importance of sterility- never use your hands
- How to change the volume

➢ Have all students change micropipette to 500ul

➢ Have students add their protein extract to the column and let it flow through

➢ As the protein is flowing, have students label 12 microcentrifuge tubes 1-12 with a sharpie
- Make sure the students align the tubes in their rack so they can easily open it and move along a conveyor like system
- Teach students to move samples over one notch to keep track of which sample they are using
- Reiterate the need for sterility and keeping tubes closed until needed

➢ Demonstrate how to elute their samples
- You may demonstrate 1-2 samples for students and let them go on their own or you can demo low salt, have them follow, and then demo high salt, have them follow
  - Add low salt buffer 1 drop at a time- collecting the elute in the tube
  - Once tube is at approximately 1ml move on to the next
  - Once 5 tubes are filled, begin adding high salt buffer one drop at a time
  - Collect 7 1ml tubes of high salt buffer
  - Label first 5 tubes "L" for low salt and last 7 "H" for high salt

- Have students compare samples between groups at the table and make some conclusions from the observations they can make of their samples
  - Can they guess which contains their protein?
  - What differences did they observe from when the extract went in and when they eluted with high salt?
  - Which samples do they think contain pure protein?

Quality Control: Product Purity

- Discuss with students the importance of quality control in biomanufacturing
  - Ask them to think of what they think biologics need to be checked for before they can go on the market
  - Purity
  - Efficacy
  - Concentration

    - *Before a bio manufactured treatment can reach a patient it must go through quality control to ensure that it is safe and functions as intended. These regulations are set by the Food and Drug Administration and it is the role of the Quality Control department in any biomanufacturing facility to ensure that all products meet these standards. One of these standards is product purity. In this part of the experiment, we are going to determine which of our eluted samples contain our purified product use spectroscopy.*

- Explain to students that to check purity we are going to use spectroscopy. Our protein product has a specific absorption and emission spectrum based on its properties. By looking at the wavelength of the emission spectra of our samples we should be able to determine if we have the right product and if there is only one product in our sample
  - Based on the spectrum given, have students make a guess, based on their results from Part I, what wavelength should be emitted by our protein
    - It should be somewhere in the blue-green
    - Ask what do they think it means if they see more than one wavelength? A different value?
  - Walk through students opening the software and creating a blank sample with water.
    - Ensure students do not touch the open sides of the cuvette as smudges will affect how light refracts and make sure they are lining up correctly in spectrophotometer
  - Work through sample 1 together to collect data
    - Micropipette sample into cuvette
    - Enter into spectrophotometer
    - Collect data
  - Have students complete the rest of their samples and determine which samples contain their protein

  - **End here for 45- minute classes**
  - *Closing Reflection*

- Close the activity with students reflecting on what they did, how it relates to biomanufacturing, any preconceptions, and careers
  - This is protein purification on a small scale- biomanufacturing is on the large scale
  - Do they think this would be ready at this point? Is it really pure?
    - Multiple steps of purification and filtration so its ready to use
    - Must still test that the protein still has its function- particularly if it was an enzyme
    - Must check concentration to ensure correct dosage
- What kind of skills/knowledge do they think it requires to work in biomanufacturing?
  - General knowledge of biology, chemistry and physics
  - Aseptic technique and documentation
  - Safety and adherence to regulations
- Did this change your view of what it is to work in biomanufacturing?
  - Did you think it was all biology?
  - Did you realize there were lots of different types of careers at every part of the process?
  - What other careers in biomanufacturing do you think there are that we didn't touch on?

- **<u>For 60 minute + Classes</u>**

- ***Protein Quantification***
- Ask students if they can tell "how much " protein they collected? Why is accuracy in the amount of product important?
  - Dosage
  - Making sure you have enough to meet needs
  - Determining the cost of process
- Explain Beer-Lambert Law- the amount of substance in a solution is directly proportional to the amount of light it absorbs or the more light it absorbs, the more concentrated it is
- We are going to use a "Standard Curve" of known amounts of protein and then compare our "unknown" concentration to that curve
- Walk through students through step 1 of creating a standard curve
  - Put 1mg/ml sample in the cuvette
  - Select absorbance vs concentration on spectrophotometer
  - Change units on axis to match sample (optional- or be sure to just students know its different)
  - Blank using a water sample
  - Measure 1mg/ml sample at 550nm
  - Hit Keep
- Have students collect their standard curve data- one standard curve can be calculated per spectrophotometer- not per group
- Once done, have students create a "best fit line" for their data by selecting the graph option and Curve Fit- Linear
- Students should record their equations.
- Ask students to look at their equation and ask them what it looks like
  - Should be an equation for a line y=mx+b

  - Have them identify y= absorbance, x = concentration and m= slope
- Students then should combine all their “pure” samples from Part 1 into a 14ml tube
- Students then read their unknown sample and collect absorbance
- Using the equation, students should calculate the concentration of their protein
  - If it is above 1, have students do a 10X dilution so that the spectrometer can read their sample. Making sure students multiply their results by 10 to get their final concentration

- ***Closing reflection (see above)***