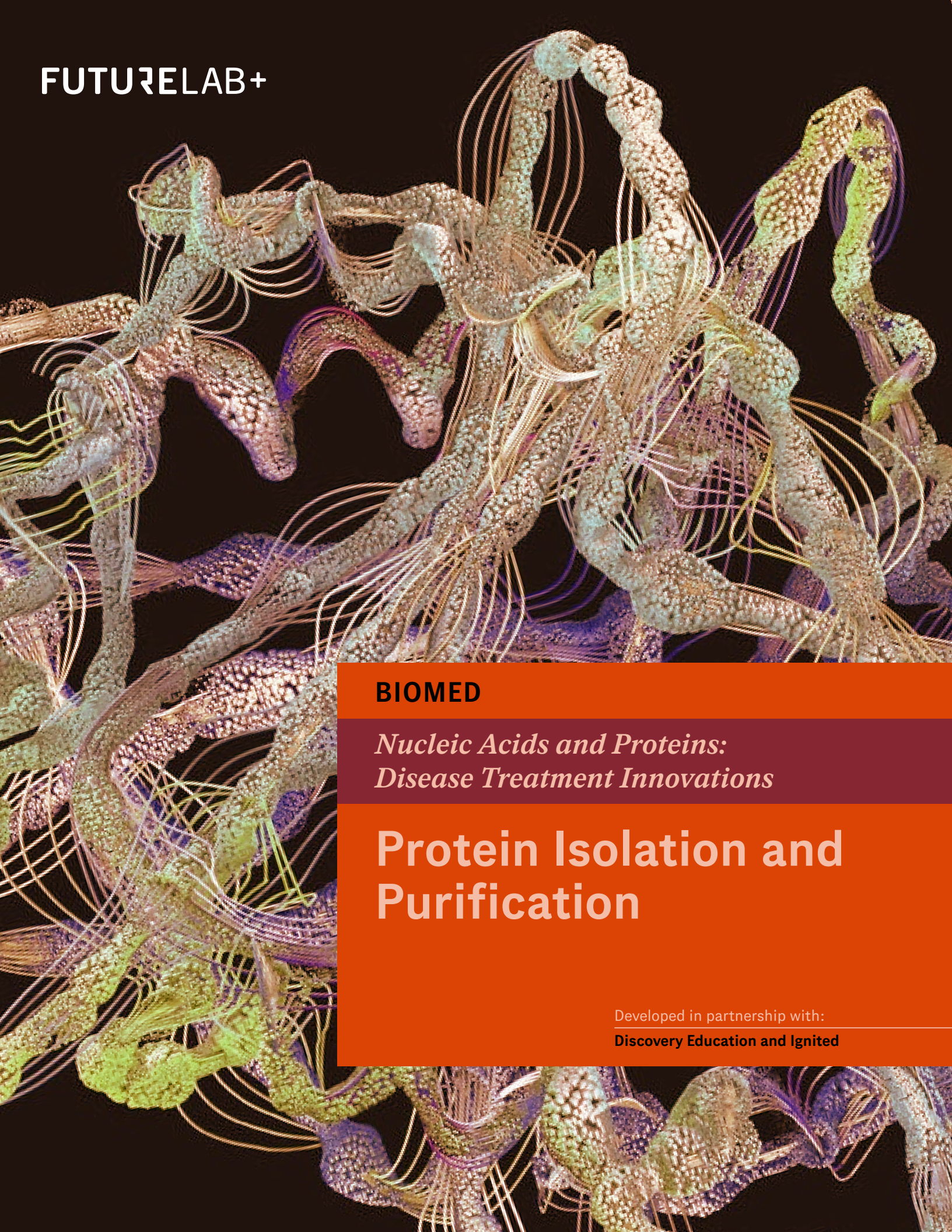
FUTURELAB+



BIOMED

Nucleic Acids and Proteins: Disease Treatment Innovations

Protein Isolation and Purification

Developed in partnership with:

Discovery Education and Ignited

In this Lesson Plan:

Print the **Teacher Section** →

Print the **Student Section** →

Cover Image
This is an illustration of a protein.

BIOMED / NUCLEIC ACIDS AND PROTEINS: DISEASE TREATMENT INNOVATIONS

Protein Isolation and Purification

ACTIVITY DURATION

Five class sessions
(45 minutes each)

DRIVING QUESTION

How are human proteins produced and isolated from non-human sources?

ESSENTIAL QUESTIONS

Why are bacterial cells used to produce some human proteins?

What are the most common cell lines or types of cells used in analytical or therapeutic biochemistry, and why are they used?

How are proteins produced and purified as part of the drug production process?

OVERVIEW

Many advances in research have been made in therapeutic medicines, making it a fast-growing industry. Biotherapeutics is an important application in biotechnology. Proteins produced using yeast, bacteria, human and other mammalian cells are used as medicines for various chronic and infectious diseases. Insulin is a biotherapeutic protein used to treat individuals diagnosed with Type 1 and Type 2 diabetes. The information in these lessons is an essential part of drug development. Throughout the lessons, students will play the role of a biochemist. The video and simulation provide students with a virtual view of the processes they would otherwise complete in a laboratory. Students will use the simulation to learn more about purifying proteins. Ultimately, students will investigate examples of proteins used in humans but isolated from nonhuman sources.

OBJECTIVES

Students will be able to:

Explain the significance of protein production and purification.

Describe the use of various protein isolation techniques.

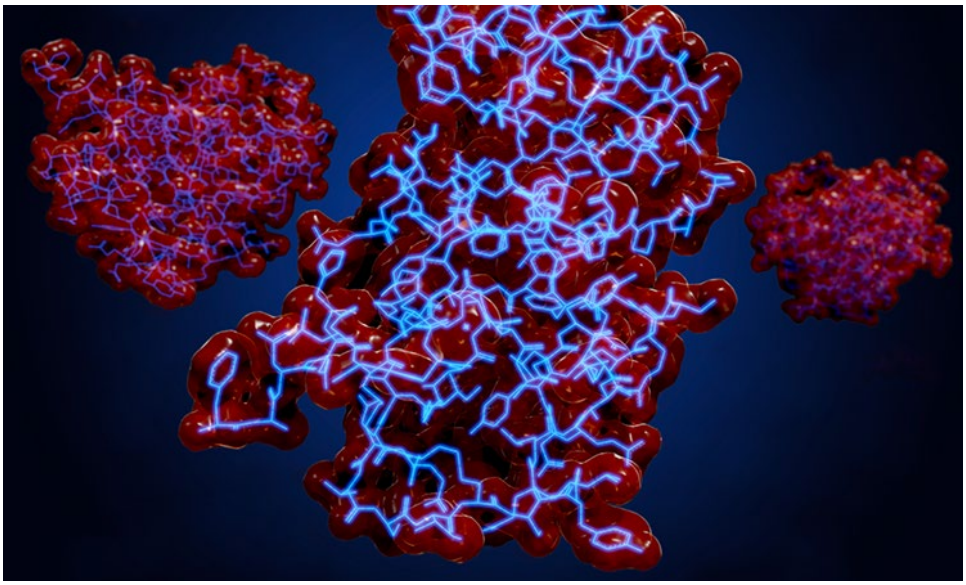
Outline the laboratory equipment needed to purify proteins.

List the various tags used in protein purification.

Investigate current examples of proteins that are used in humans and isolated from nonhuman sources.

BACKGROUND INFORMATION

Students should be able to describe how DNA is used to create a final protein. They should also be familiar with protein modifications to understand the limitations of some protein production methods. The aforementioned topics are discussed in previous lessons in this unit. Students also should be familiar with certain equipment used in the lab, such as centrifuges, gel electrophoresis, and agar plates.



Materials
Aluminum Foil
Beads
Cotton Balls
Scoring Tool
Computers
Rectangular Sheets of Bulletin Board or Poster Board
Marker
Plastic Cups
Modeling Clay
Paper Stars
Pipe Cleaners
Plastic Pieces (any shape)
Craft Stick
Puff Balls
Cotton Swabs

Materials (cont.)
Rubberbands
Scissors
Tape
Tissue
Toothpicks
Empty Toilet Tissue and Paper Towel Rolls
The Product Cycle of Medicine Diagram
Diabetes and Insulin Tabletop Jigsaw Activator
Diabetes and Insulin Tabletop Jigsaw Capture Sheet
Methods of Protein Production Capture Sheet
Techniques in Protein Production Review
Small Group Alternative: Sorting Cards

Materials (cont.)
Protein Purification Techniques Video Capture Sheet
Protein Purification Lab Tools and Protein Tags Capture Sheet
Protein Characteristics List
Protein Purification Flow Chart Assignment
Protein Purification Flowchart Rubric
Protein Purification Lab Manual Assignment
Protein Purification Lab Manual Rubric
Protein Purification Review
Nonhuman Proteins Used in Humans Assignment
Nonhuman Proteins Used in Humans Rubric
Design Journal

Pedagogical Framing

Instructional materials are designed to meet national education and industry standards to focus on in-demand skills needed across the full product development life cycle—from molecule to medicine—which will also expose students and educators to the breadth of education and career pathways across biotechnology.

Through this collection, educators are equipped with strategies to engage students from diverse racial, ethnic, and cultural groups, providing them with quality, equitable, and liberating educational experiences that validate and affirm student identity.

Units are designed to be problem-based and focus on workforce skill development to empower students with the knowledge and tools to be the change in reducing health disparities in communities.

SOCIAL-EMOTIONAL LEARNING

Because students will work in group settings, self-awareness of their contribution to the group as well as their self-management behavior towards their group members is important. Students also will discuss and research certain diseases that they may have experience with and this will allow for the practice of empathy toward one another.

CULTURALLY AND LINGUISTICALLY RESPONSIVE INSTRUCTION

The lesson offers opportunities for the growth of critical consciousness of self and community, while encouraging students to bridge the learning to their real world experiences. Students discover challenges for the manufacturing and use of medicines. Students discuss what they believe are important factors in determining if a drug should be created for a particular disease. They note that tropical diseases in African countries are oftentimes not a focus for research and development of medicines. Students discuss issues of social justice with the use of recombinant proteins for therapeutics. They discuss affordability and access to these proteins.

ADVANCING INCLUSIVE RESEARCH

There is still much to be learned about proteins, and how the differences in our DNA affect the ways our bodies make them. Most of the genetic information available for scientific study comes from white populations. It is important to expand our pool of knowledge on how different bodies produce proteins by expanding research into BIPOC communities.

COMPUTATIONAL THINKING PRACTICES

This lesson focuses on protein production, which is one way treatments and therapies are developed to treat diseases. Protein production, and the Product Cycle of Medicine, are two examples of the computational thinking strategy of developing algorithms, which involves creating step-by-step instructions on how to complete a task. Students gain experience with the computational thinking strategy of developing algorithms as they create a flowchart outlining the steps of protein production. Elsewhere in the lesson, students practice the computational thinking strategy of collecting data as they learn about different cell types, diseases, and possible ways to modify proteins.

CONNECTIONS TO THE PRODUCT LIFE CYCLE

In this lesson, students are invited to dig into the product cycle of medicine in order to develop context for why protein isolation and purification are practiced. As they do this, students simulate the product cycle and gain an understanding of why the cycle involves a pyramid, starting with a wide data collection process and narrowing down to a commercialized medicine.

Have you ever wondered…

How medicines like insulin are made?

Individuals who have Type 1 Diabetes can be treated with a medicine known as insulin. A human gene and non-human cells are used to make most insulin. Insulin is mainly manufactured using bacteria or yeast.

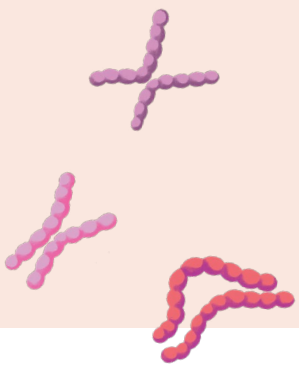
How do scientists know that a biotherapeutic will work?

Scientists use genetic information from a database to determine whether a certain drug would be an effective target. Additional research in the form of assays are done to ensure the biotherapeutic successfully works in cells.

MAKE CONNECTIONS!

How does this connect to the larger unit storyline?

Students will gain an understanding of the use of a variety of protein purification methods. They will use this knowledge to explain advantages and disadvantages of using one purification method over another. Ultimately, this information is essential to know as it must occur prior to a protein being used as a drug.



How does this connect to careers?

Biochemists use different techniques in the lab to achieve a final goal. They are experts at deciding when and what procedure to use for a specific goal. Biochemists play an essential role in the product cycle of medicine. They can determine the efficacy of a drug that will be possibly made for therapeutic use.

How does this connect to our world?

The need for therapeutic products to be created is steadily increasing. Techniques used to assist with the treatment of many ailments rely on therapeutic products created in the lab. This industry requires scientists who can think critically and make informed decisions on a wide variety of protocols to be used in the lab. Well-designed protocols minimize cost and waste and can improve the health status of many around the world.

Day 1

LEARNING OUTCOMES

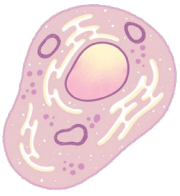
Students will be able to:

Explain the significance of protein production and purification.

Describe protein production techniques that use CHO, HEK-293, bacterial, yeast, and plant cells.

COMPUTATIONAL THINKING IN ACTION

In this activity, students use the computational thinking strategy of collecting data to learn about protein production. The scaffolded approach of data collection (first examining stations different types of cells, then completing a Jigsaw about diabetes and the protein enzyme insulin, and finally building to the product life cycle for drug manufacturing) uses the computational thinking strategy of decomposition to explore the fundamental components of drug development.



Procedure

Teacher Note > *On this day, students are going to explore the various techniques used to produce and purify proteins. These techniques require different cell lines and students will acquire information about these cell lines by reading college-level scientific papers and abstracts. To ensure the high reading level of these papers and abstracts does not impede student tasks, make heterogeneous reading groups. Emphasize to students that the images in each of the articles can help complete the table in the capture sheet.*

Whole Group (10 minutes)

1 **Advance Preparation:** Prior to student arrival, set up six stations around the room. Print out the articles or abstracts found at each of the links for each station (below), and place them at each of the appropriate stations. Label each of the stations as Station 1, Station 2, etc.

- **a.** Station 1: *CHO cells*
- **b.** Station 2: *HEK-293 cells*
- **c.** Station 3: *Bacterial cells*
- **d.** Station 4: *Yeast cells*
- **e.** Station 5: *Plant cells*
- **f.** Station 6: *Significance of protein production and purification*

2 To provide a purpose for learning, students will be working through the *Diabetes and Insulin Tabletop Jigsaw Activator* to learn more about diabetes mellitus. A student capture sheet is provided but completing this on an electronic device would be best because the resources are links. Each group will have just one or two questions to answer and will share what they have learned with the rest of the class. If there are white boards, students could write the answer on the white board to share; otherwise, students can answer on their paper. Give the students three minutes to find the answer.

3 Have each tabletop group share their answers. Go in order from table 1 to 8 as one question leads to the next. Students can record the answers on their paper if they wish, however it is not necessary. Students who wish to learn more about diabetes can use the links on their own as an extension.

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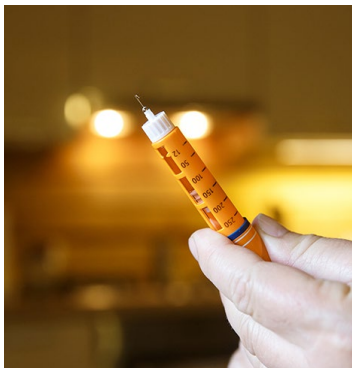
Day 1

Continued



Procedure

4	Discuss with students why diabetes is a global epidemic. Emphasize that many people with diabetes are able to live a better life because of insulin. Tell students that insulin is a protein enzyme that can be made in the lab. Let them know that protein production and purification in the lab play an important role in creating medicines, such as insulin, for therapeutic use.
5	Tell students that the ability to produce proteins enables scientists to determine if they possibly could be used as a medicine to treat a particular disease. This would be included in the discovery phase of the product cycle of medicine, specifically identifying a drug target.

Teacher Note > *Project The Product Cycle of Medicine Diagram, so students are able to view and answer questions.*

6	Ask students to share what they believe are important factors in determining whether or not a medicine is created for a disease. Let students know that research and development in medicine does not take place at the same pace for all diseases. (Display the image of the picture of the product cycle of medicine on the overhead screen for students to use as a reference.) In particular, medicines for diseases affecting individuals living in less technologically or infrastructurally advanced countries are slow to be manufactured. Discuss with students some examples of diseases that are common in under developed countries.
7	Tell students that they will be learning about the various techniques that are used to produce and purify proteins, like insulin, in the lab so that they can be used as medicines.

INDUSTRY AND CAREER CONNECTIONS

During this activity, student soft skills include attention to detail and time management. Let students know that much like a biochemist they will need to read the various sources in order to find the important information. Because they only have six minutes at each station, they will need to use this time wisely. It would be in the best interest of students to assign tasks for each group member, including information from the chart that needs to be located.

Small Group (35 minutes)

1	Split students into groups of three or four. Let students know they will be completing a scavenger hunt using information from six different stations around the room.
2	Hand out the *Methods of Protein Production Capture Sheet* to each student.

Continues next page >

Day 1

Continued

Procedure

3 Describe the information students will find at each station (refer to Whole Group instruction notes). Have them label their capture sheet with the name of each of the stations.

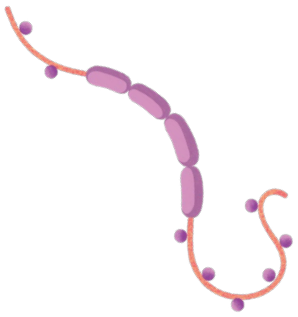
Characteristics	CHO/HEK-293/Bacterial/Yeast/Plant Cells
Prokaryotic or Eukaryotic	
Description (What is the full name? What is it used for?)	
Advantage	
Disadvantage	

4 Let students know that they will be using a variety of sources in order to complete a scavenger hunt on two techniques used in protein expression and isolation. Emphasize that students should start at the first two resources in order to locate the description.

5 Also let students know to focus on the information that can be found in the abstract before reviewing information in the article.

6 Give students six minutes at each station. Keep a running timer on the board using: *A Free Countdown Timer*. An alert should be sounded when one minute is left at a station.

7 Collect capture sheets at the end of class.



Day 2

Procedure

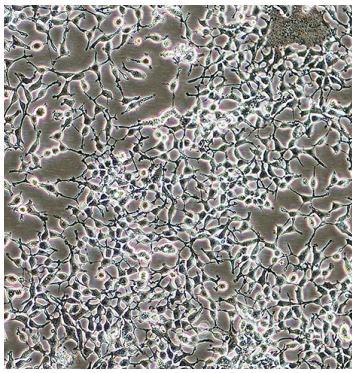
LEARNING OUTCOMES

Students will be able to:

Distinguish among cell types used to produce proteins.

Describe the importance of protein properties in the purification process.

Outline the laboratory equipment needed to purify proteins.



Microscopic Image showing EK293 cells in cell culture flask.

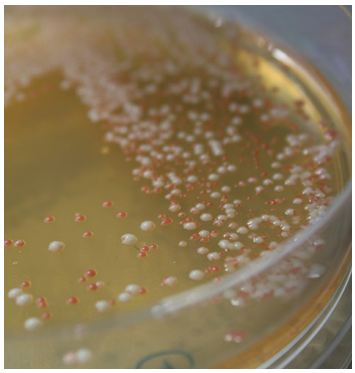


Image shows yeast colonies growing on an agar plate.

Teacher Note > *Be sure to hand back each student's work so they will be able to use it to help them answer the questions on the review capture sheet, Techniques in Protein Production Review. For the protein purification cup activity, try to use something magnetic as one of the three proteins. Students can fold the long rectangular pieces of paper accordion style. Students also can create their own style of flowchart as long as it resembles the one located here.*

Whole Group (2 minutes)	
1	Notify students that today they will be reviewing the information learned in the last class. In addition, they will be discussing methods used to purify proteins.
2	Provide students with the *Techniques in Protein Production Review Capture Sheet*.
3	Let them know that they can use the information gathered from the station work to complete the capture sheet.

Individual Work (15 minutes)	
1	Give students 10 minutes to complete the *Techniques in Protein Production Review Capture Sheet*.
2	In the last five minutes, review the most commonly missed answers. Call on students to *Stand and Share* their answers. Also ask students if they have any questions.

Teacher Note > *Walk around and note which questions students may have. These are the questions that can be focused on during the review. You may want to print them on card stock or laminate the cards.*

Continues next page >

Day 2

Continued

Procedure

Small Group Alternative (15 minutes)

Teacher Note > *If you would like to encourage more student discourse, you could use a Sorting Cards activity instead. You will need to print and cut eight groups of cards and place them in an envelope ahead of time.*

1	Ask students to work with their tabletop group to sort the cards using each method cell type. Some cards have the same clue which means that it will match with more than one cell type. The number of matches for each cell type is indicated on the cell type card.
2	Walk around to each group and assist students by removing cards that are not placed correctly. Some groups may need a clue to help them make the match. This may also give you an opportunity to discuss or clarify any information.
3	Once all cards are sorted, have students check their answers by displaying the correct sort as shown in the *Small Group Alternative: Sorting Cards Answer Key*.
4	Next, each group should come up with their own clue and write it on the seven blank cards. Have one student from each group deliver their clue card to all of the other tables. Each group should try to match the clue with the correct method.
5	After a few minutes, each group should read their clue and have every other group hold up the card with the correct cells. The group should reveal the correct answer.

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Day 2

Continued

Procedure

COMPUTATIONAL THINKING IN ACTION

This lesson focuses on the Product Cycle of Medicine, which is an example of the computational thinking strategy of building algorithms. Algorithms are step-by-step sets of instructions on how to complete a task—in this case, getting a drug to market.

Whole Group (15 minutes)

1. Let students know that they will spend the next 15 minutes of class learning about how to purify the protein. Emphasize that students have so far learned about the various methods of protein production, but these proteins now need to be isolated to be able to be used as a drug. Show students the *The Product Cycle of Medicine Diagram*.

2. Ask students, "Which portion of the product cycle of medicine would protein purification be associated with?" Remind students that this step might include the discovery phase in which scientists are experimenting with the protein as a potential drug or for clinical trials. This phase takes place at the beginning of the Product Cycle of Medicine.

3. Hand out the *Protein Purification Techniques Video Capture Sheet*.

4. Have students review the video found here: *Methods for Protein Purification*.

5. *Pause at various moments* during the video to ensure students are recording appropriate information on their capture sheet:

   a. 0:00–2:00: answers #1, 2, and 3

   b. Review student answers. Call on a few students to share their answers.

   c. Play video from 2:50–4:00: answers #4–5

   d. Play video at 7:07—pause the video at the cartoon. Emphasize the different properties of each of the proteins, some negatively charged, some large, some small, etc.

   e. Continue playing video until 8:07. Have students complete #6.

   f. Play video from 8:07–8:50.

Continues next page >

Day 2

Continued

Procedure

g. Let students know that for the last portion of class, they will be working in groups of three as a team of biochemists. Reiterate the challenge: You will be tasked with separating three different proteins from a mixture. Emphasize that students cannot use their hands and can only pour the contents of the cup out or pour things into the cup.

h. Make it clear that students are able to use three different tools including a sieve, a funnel, water, magnet, and cups. Also let them know that the tools or different methods cost both time and money. Let them know that scientists compare variables such as protein quality and protein yield with a technique's cost and labor when attempting to decide which methods to use to purify proteins.

i. Put students in groups of threes. Provide each group with a cup of the three proteins (paper dots, puff balls, and small plastic pieces).

Small Group (13 minutes)	
1	Monitor students as they work, noting any challenges or similarities groups have.
2	Remind students of the challenge of separating the three different types of proteins present in a cup on their tables without using their hands to directly touch the contents in the cup.
3	Remind students to write down their idea as a flowchart on pieces of an accordian-folded piece of notebook paper. Each step in their flowchart will be placed in a separate section or fold. Refer students to the *flowchart* used to describe how insulin is created.
4	Explain to students that they should include the property of the proteins (dots, balls, or pieces) and how the differences in these properties can form the basis for the technique chosen to separate the proteins from one another.
5	Have students turn in their flowchart at the end of class.

Day 3

LEARNING OUTCOMES

Students will be able to:

Explain the types of methods used in protein purification.

List the steps of the protein separation process.

Identity common tags used in protein purification.

Understand the purpose of protein tags.

INDUSTRY AND CAREER CONNECTION

Throughout this lesson students will be working as biochemists. Biochemists' technical skills include knowing basic lab skills. They also need to know math and how to analyze and interpret data. Soft skills include organization—most biochemists use a lab notebook while performing experiments in the lab. This helps them stay organized and remember what research was completed each day. Students will need to be organized and detail-oriented because they will be gathering information from the simulation.

Procedure

Whole Group (20 minutes)	
1	Have at least three student groups volunteer to explain their flowcharts from last class.
2	Play *Methods for Protein Purification* from 9:05 until 10:52 minutes.
3	After playing the video, acknowledge students whose flowcharts described similar techniques as shown in this section of the video. Also, acknowledge how other group ideas could also work.
4	Tell students that they will work in these same groups for the next activity. Remind them of the segments of the video they watched in the previous class. Ask them if they remember the different characteristics of proteins (charge, solubility, size).
5	Hand out the *Protein Purification Lab Tools and Protein Tags Capture Sheet* to each student. Let students know that they will be completing the table in the capture sheet as you walk them through the various methods.
6	Tell students that today they will learn about different techniques of protein purification.

Continues next page >

Day 3

Continued

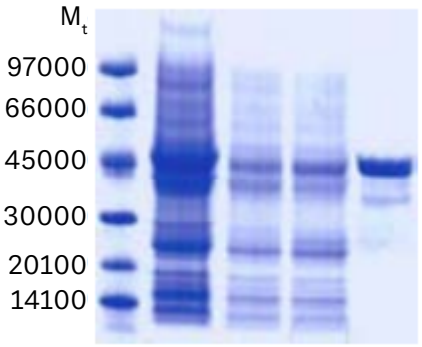
Procedure

7 Review each of the protein purification methods in the table below.

Protein Purification Lab Tool	SDS–Page	Affinity Chroma-tography	Hydrophobic Interaction Chroma-tography	Gel Filtration	Ion Exchange
Rationale For Its Use	Use to make sure the correct protein was purified (size)	Use to make sure a specific protein is purified	Use if desired protein is hydrophobic	Use when desired protein size is known	Use when desired protein charge is known
	Use with ladder of known molecular weights	Antibodies or other molecules can bind to protein	Separates based on solubility (attraction to water)	Separates based on molecular size	Separates based on charge

8 Show the images of each process as you walk through each one:

a. SDS-PAGE: Each lane represents different time periods in the protein purification process. Ask students which lane represents the purified protein (the last lane)



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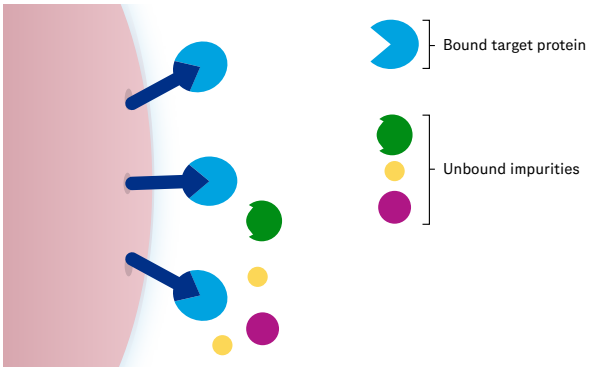
Day 3

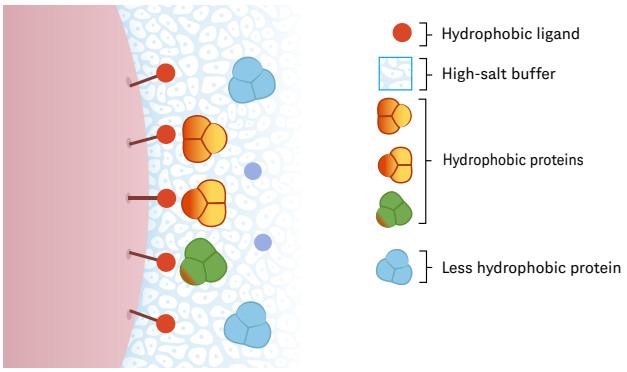
Continued

Procedure

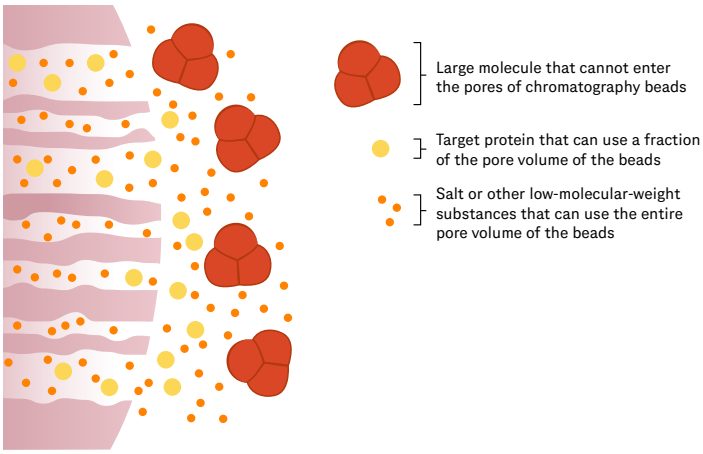
b. Affinity Chromatography—ask students what affinity means. (a natural liking for something or someone)



c. Hydrophobic Interaction



d. Gel filtration



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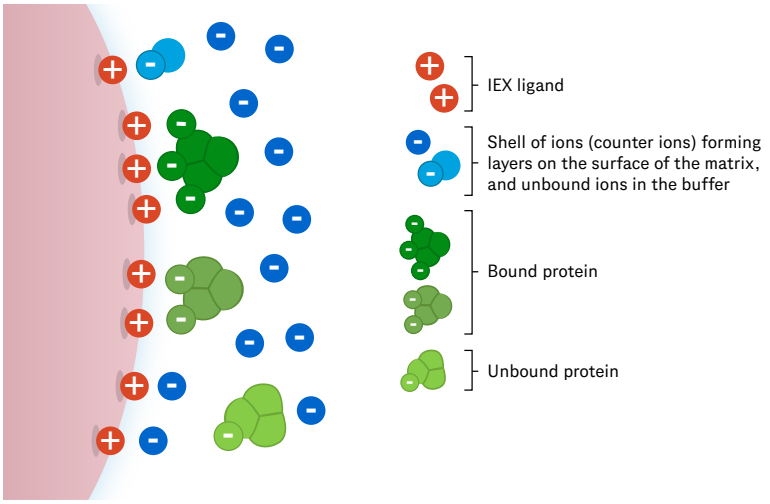
Day 3

Continued

Procedure

e. Ion Exchange



Whole Group (15 minutes)	
1	Share that students will now review another method scientists use to help purify a protein.
2	Let students know that similar to how they completed the table in *Protein Purification Lab Tools and Protein Tags Capture Sheet*, they should now be able to explain the reasoning behind the use of a certain piece of equipment.
3	Give students two minutes to complete the scramble portion of the capture sheet.
4	Review the answers with students.
5	Let students know that they will be learning about protein tags, which can also be used in the process of purifying proteins. Inform them that these tags help further ensure that the correct protein is purified. Create an analogy for using protein tags in the purification process using the protein separation activity students completed with the cup during Day 1. If one of the proteins had a certain protein tag, it could be purified by using a method that specifically identifies this tag.

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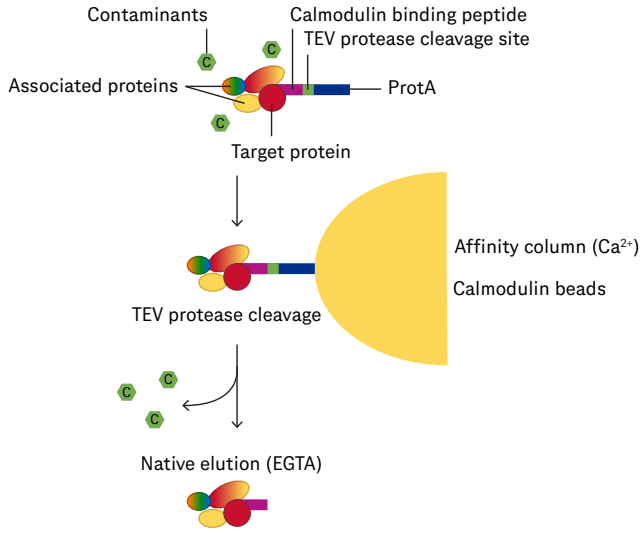
Day 3

Continued

Procedure

6 Show the image:



7 Tell students to follow along on the capture sheet as you talk.

8 Let students know that during the purification process, protein tags can be used. Share with them that protein tags are another example of affinity chromatography. Ask them to complete their notes with this: Protein tags are different from antibodies in that they are not specific to the protein. Ask students how else they may be different (price, quality of output, or protein). Tell them to note the following on their paper in the description box provided.

a. Describe the model in the image:

i. Purification of the target protein is the goal (point to the target protein at the top and the native elution, or purified protein, at the bottom).

ii. The green contaminants need to be removed.

iii. The affinity column is made of protein tags known as calmodulin beads.

iv. Calmodulin beads recognize proteins that have a calmodulin binding site such as proteins belonging to the families of ribosomal proteins, proteasome, and deubiquitinating enzymes. Gauge student memory on functions of ribosomal proteins, proteasome, and ubiquitination.

Continues next page >

Day 3

Continued

Procedure

9	Provide students with the analogy of the purification process being like the target protein having a ticket that the calmodulin bead accepts. Similar to the other processes, this removes molecules that do not "fit" the particular criteria, in this case having a calmodulin binding domain or site, which attaches to the "binding bead."
10	*Display Table 9.9.1* on the projector or screen. This table includes a list of commonly used protein tags along with their characteristics, including use.
11	Walk through a specific example using calmodulin binding peptide (CBP) with students as they complete the table.
12	Zoom in to the comments section on CBP. Ask students what type of nonhuman protein production would be best to use CBP. (prokaryote because they do not have any molecules that will bind to CBP and eukaryotic cells would not be used due to the idea that CBP may bind to other molecules besides the target.)
13	Repeat for His-Tag.
14	Ask students what are the benefits of using green fluorescent protein (GFP) as a tag. (Protein can be visualized using a microscope. Scientists can study the protein and its location in cells or a particular tissue or organ.)
15	Scroll to GFP. Ask students what type of experiments scientists are doing when they use GFP. Emphasize to them their inability to purify proteins. (experiments where they want to visualize the movement or location of a protein)
16	Let students know that for the last 10 minutes of class, they will summarize the notes from today's lesson in their **Design Journal**.
17	Tell them that they have the option of summarizing their notes in a paragraph or as a bulleted list.

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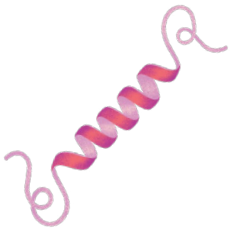
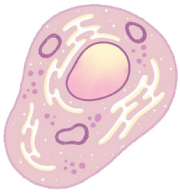


Day 3

Continued

Procedure

Individual (10 minutes):	
1	Students will spend time summarizing their notes in a form of their choice. They may choose to create a word collage, images with captions, or another method of their choice.
2	Monitor students as they work.
3	Ensure students are including correct vocabulary and any relevant images.



Day 4

LEARNING OUTCOMES

Students will be able to:

Describe methods of protein purification.

Identify lab tools used to purify proteins.

Procedure

Teacher Note > *For this day's activity, students need to fold long rectangular pieces of paper in accordion style. Students will use this to create a protein purification flowchart. Students can also create their own style of flowchart as long as it resembles the one located here. In addition, students will be tasked with preparing a protein purification lab manual for trainees.*

Individual Work (3 minutes)	
1	**Advance Preparation:** Copy the *Protein Characteristics List* and cut into strips, enough for every student to have one.
2	Let students know that they will now create a flowchart to model the protein purification technique they would use for a randomly chosen protein.
3	Pass out the *Protein Purification Flowchart Assignment*.
4	Let students know that they will need to not only describe the steps, but also explain the reasons why they chose a particular protein purification method for their assigned protein.
5	Let them know that they will be randomly choosing a protein that includes a few characteristics that should assist them with deciding which protein purification method to use.
6	Hand out long rectangular pieces of paper and have students choose the protein characteristics from the paper bag.

Small Group (20 minutes)	
1	Give students 20 minutes to work on their flowcharts.
2	At the end of 15 minutes, let students know that they will now be explaining their rationale to another classmate.
3	Let students know that they will meet with two additional students to discuss their flowchart and rationale.
4	Tell students that they will have 45 seconds to explain their flowchart to their shoulder partner.

Continues next page >

Day 4

Continued

Procedure

5	The other student has 15 seconds to agree or disagree with the choice and provide reasoning.
6	After one minute, have students find another partner and repeat.

Whole Group (2 minutes)	
1	Let students know that they will now design a lab manual for trainees on the various methods of protein purification.
2	Hand students the *Protein Purification Lab Manual Assignment*.
3	Review the assignment details.
4	Let students know that they will be working in small groups and each member of the group will be responsible for including a different protein purification technique in the lab manual. Split students into groups of three or four students.
5	Assign each student one of the protein purification methods listed on the paper: hydrophobic interaction chromatography, affinity chromatography, gel filtration, isoelectric or ion exchange.

Teacher Note > *Be sure to not assign the same method to students working in the same group. Each group member should have a different purification technique, which should be represented in the lab manual.*

Small Group (20 minutes)	
1	Monitor students as they work to respond to questions and support students as needed.
2	Remind students about pacing and prioritizing certain requirements of the assignment, such as the model.
3	Let them know that they will need to finish the rest of the assignment for homework.

Day 5

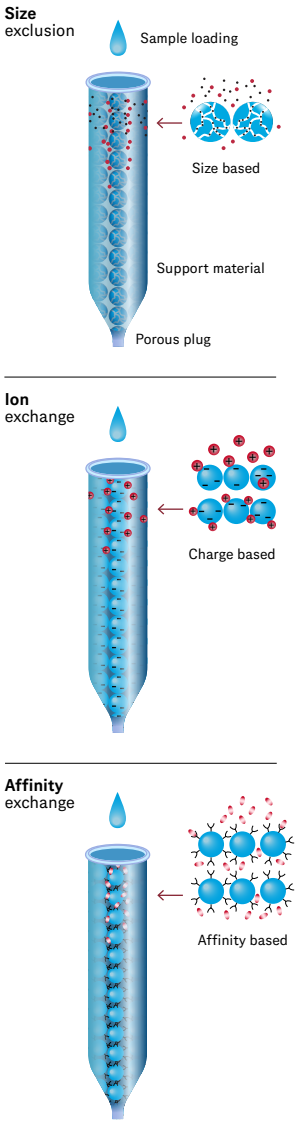
LEARNING OUTCOMES

Students will be able to:

Distinguish between methods of protein purification.

Outline the use of a nonhuman protein that will be used as medicine for a human disease.

Chromatography Types



Procedure

Individual Work (15 minutes)

1. Provide students with the *Protein Purification Review Capture Sheet*.
2. Let students know they will have 10 minutes to complete the capture sheet.
3. Tell them they can use their flowchart and the information learned in class yesterday to help them answer the questions.
4. As students work, monitor and gauge for student understanding or any misconceptions.

Whole Group (5 minutes)

1. Tell students that for the rest of class they will work on a project that will summarize all of the information learned this week.
2. Provide students with and explain the components of *Nonhuman Proteins Used in Humans Assignment*.
3. Let students know that they will each choose a protein that is currently used in humans. Examples include human growth hormone or insulin.
4. Gauge student opinion by asking them if:
   - **a.** a recombinant protein should only be used for therapeutic use (ie., human growth hormone only used if issue is growing—not used if the person is already of normal size and only wants to enhance)
   - **b.** the price for a therapeutic protein should change depending on your income level (ie., people with lower incomes will pay a lower price than those with higher incomes); remind students that the labor and costs to make the drug is the same
5. Review the assignment details.

Continues next page >

Day 5

Continued

Procedure

Individual Work (25 minutes)	
1	As students work, remind them to use reputable sources.
2	Provide them with a time check.
3	As a wrap-up, have students respond to lesson questions in their **Design Journal**. They will consider the most common cell lines or types of cells used in analytical or therapeutic biochemistry and how proteins are produced and purified as part of the drug production process.

Teacher Note > *The following links provide opportunities for exploration into academic research:*

Recent Developments in Bioprocessing of Recombinant Proteins Expression Hosts and Process Development

Where Does Medicine Come From

Pharmaceutical Manufacturing: Current Trends and What's Next

National Standards

Next Generation Science Standards

Science and Engineering Practices

Obtaining, evaluating, and communicating information

Critically read scientific literature adapted for classroom use to determine the central ideas or conclusions and/or to obtain scientific and/or technical information to summarize complex evidence, concepts, processes, or information presented in a text by paraphrasing them in simpler but still accurate terms.

Crosscutting Concepts

Scale, Proportion, and Quantity

Algebraic thinking is used to examine scientific data and predict the effect of a change in one variable on another (e.g., linear growth vs. exponential growth).

Systems and System Models

Models (e.g., physical, mathematical, computer models) can be used to simulate systems and interactions—including energy, matter, and information flows—within and between systems at different scales.

Career and Technical Education (CTE)

A3.4

Predict outcomes of DNA and protein separation protocols.

A5.2

Predict outcomes of DNA and protein separation protocols.

A8.1

Follow written protocols and oral directions to perform a variety of laboratory and technical tasks.

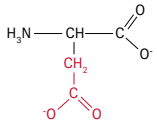
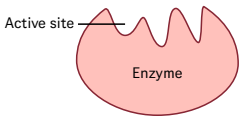
A9.1

Describe the major steps of a product's move through a company's product pipeline.

Educator Resources

Protein Characteristics List

These ten different proteins can be duplicated for the number of students in the class. Cut into strips and place in container for students to randomly select their protein.

Protein A	MW = 800 bp; ph: 8; soluble in water
Protein B	MW = unknown; ph: unknown; cation
Protein C	MW = unknown; is glycosylated
Protein D	MW = 3.2 kb; hydrophobic
Protein E	MW = unknown; contains Histidine Binding Domain
Protein F	
Protein G Product Cycle of Medicine	MW = 7.5 kb; ph: 6; hydrophobic
Protein H	MW unknown, pH: 2
Protein I	MW = 5 kb
Protein J	

Educator Resources

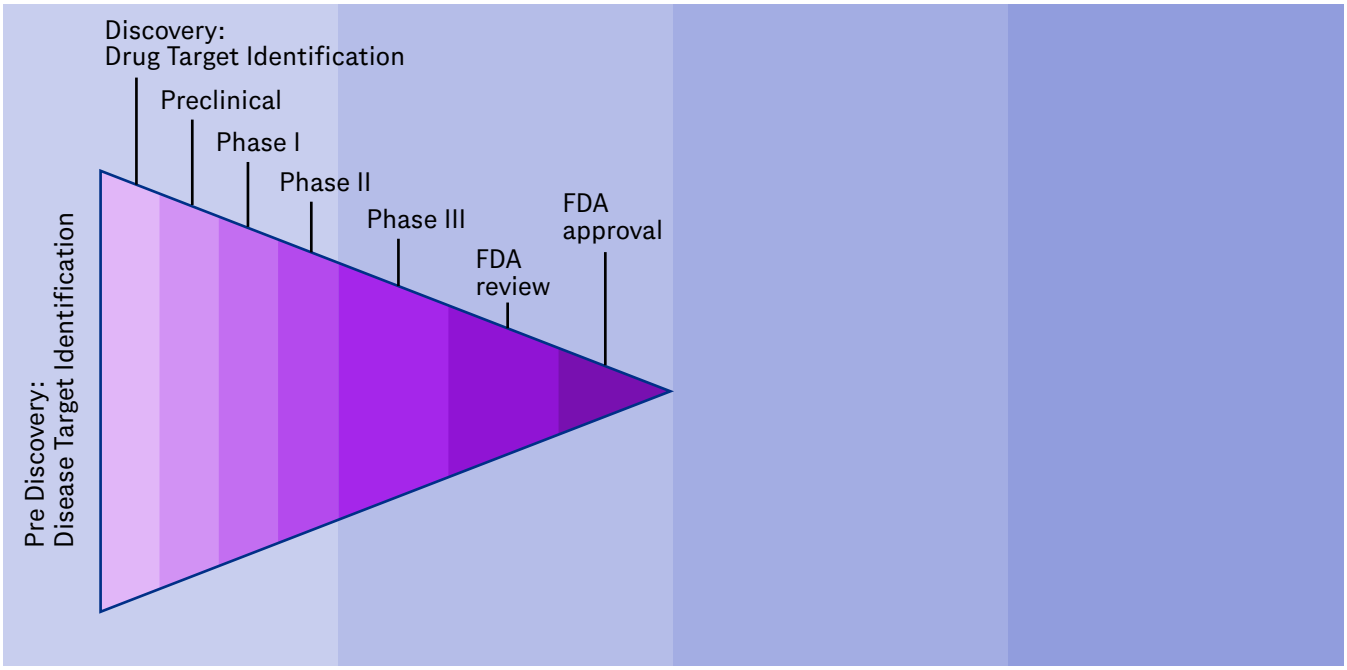
Product Cycle of Medicine Diagram

Discover
Includes:
Early stage research, late stage research, early clinical trials

Develop
Includes:
Stage III clinical trials, FDA review and approval

Manufacture
Includes:
Technical operations

Commercialize
Includes:
Commercial, medical, and government affairs



Jobs include:
- Research
- Developmental Science
- Early Clinical Development
- Project Management and Operations
- Partnering

Jobs include:
- Biometrics
- Clinical Operations
- Clinical Science
- Personalized Healthcare
- Portfolio and Product Development Strategy
- Medical Affairs
- Quality
- Regulatory
- Safety Risk Management

Jobs include:
- Innovation, Technology and MSAT
- Pharma Technology Regulatory
- Product and Supply Chain Management
- Quality and Compliance
- Global Engineering and Facilities
- Drug Product Manufacturing
- Global Technical Development
- Strategy Office

Jobs include:
- BioOncology
- Opthamology and Immunology
- IMPACT
- Business Operations
- Managed Care and Customer Operations
- U.S. Medical Affairs
- Government Affairs

Jobs include:
- Science Communications
- Bioethics

Diabetes and Insulin Tabletop Jigsaw Activator

ANSWER KEY **Do not share with students**

Directions
Use the link provided to answer the question(s) that follow. Work with your tabletop group to agree on the best answer. Be prepared to share your answer with the class.

Group 1

Diabetes: Types, Risk Factors, Symptoms, Tests, Treatments & Prevention

What is diabetes?

Diabetes Mellitus is a chronic (long-lasting) condition in which your body does not make enough insulin or it can't use the insulin that it makes as well as it should. It results in high blood sugar levels. If left untreated, high blood sugars can cause serious complications, such as problems with eyes, kidneys, nerves, and the heart.

Group 2

A Snapshot: Diabetes In The United States | Diabetes

Use the infographic to answer the following question:

Why is diabetes a problem in the United States?

There are many acceptable answers to this. It could be that 1 in 10 people have diabetes and 1 in 5 people don't know that they have diabetes. Also, 1 in 3 people in the United States are prediabetic. The risk of death is 60% higher for people with diabetes and the costs associated with treating diabetes are very high.

Group 3

A Snapshot: Diabetes In The United States | Diabetes

Diabetes: Types, Risk Factors, Symptoms, Tests, Treatments & Prevention

What is the difference between Type 1 and Type 2 diabetes?

Type 1 diabetes is when your body is not making enough insulin. It can develop at any age and there is no known way to prevent it.

Type 2 diabetes is when your body cannot use the insulin that is made. It too can develop at any age but can be prevented through healthy living such as diet and exercise.

Group 4

US Diabetes Surveillance System

What has happened to the number of individuals in the United States with diabetes in the last 18 years?

The percent of Americans with diabetes increased from 5.9% 2000 to 10.1% in 2018.

Click on your state.

What percent of the population in your state have been diagnosed with diabetes?

How does this compare to the national level?

Answers will vary depending on the state.

Continues next page >

Diabetes and Insulin Tabletop Jigsaw Activator

ANSWER KEY **Do not share with students**

Do not share with students

Continued

Group 5

A Snapshot: Diabetes In The United States | Diabetes

What is the best way to treat or prevent diabetes?

The best way to treat diabetes is to lose weight if needed, eat healthy, and be more active.

Group 6

Diabetes: Types, Risk Factors, Symptoms, Tests, Treatments & Prevention

What is insulin?

Insulin is a protein hormone that is released from the pancreas when blood sugar increases. It helps to allow sugar to enter cells.

Group 7

Insulin and Diabetes Management Insulin

Where did the first commercially produced insulin come from?

The first insulin was extracted and purified from the pancreas of pigs and cattle.

What important event happened in the 1980s, in regards to insulin production?

The demand for insulin was higher and the first "recombinant insulin" called Humulin was made. The recombinant DNA lab technique allowed manufacturers to genetically engineer human insulin using bacteria.

Group 8

Insulin and Diabetes Management Diabetes Remedies Before Insulin

What was the most effective treatment for diabetes before insulin?

Treatment was highly restricted diets with very few carbohydrates. They were the so-called starvation diet or under-nutrition treatment.

Methods of Protein Production Capture Sheet

ANSWER KEY — **Do not share with students**

Directions
Use the information located at each station to complete the chart. You will have six minutes at each station. Use the first two resources to find descriptions of each method. Choose which group member will locate specific information from the chart.

Note >
Use the Interesting fact section in the answer key below to augment the lesson while students are working and during the review.

Station 1: CHO Cells

CHO cells in biotechnology for production of recombinant proteins: current state and further potential

CHO cells can make more protein

Cell Type	CHO Cells
Prokaryotic or Eukaryotic	Eukaryotic
Description *What is the full name? What is it used for?*	Chinese hamster ovary cells—used to express and produce large amounts of recombinant proteins aka biopharmaceuticals
Advantage	Produces 10g/L of protein Extensive characterization (know a lot about them) A lot of regulatory approval
Disadvantage	Cannot perform post-translational modifications; produces molecules not expressed in humans
Interesting fact	Most commonly used mammalian host for large-scale commercial production of therapeutic proteins—used to make over half of all therapeutic proteins on the market Worldwide sales from products made by CHO cells exceed $140 billion.

Station 2: HEK-293 Cells

A Multi-Omics Analysis of Recombinant Protein Production in HEK-293 Cells

Cell Type	HEK-293 Cells
Prokaryotic or Eukaryotic	Eukaryotic
Description *What is the full name?*	Human embryonic kidney cells
Advantage	Can perform post-translational modifications; can produce proteins that are similar to those produced naturally in humans; easily grown
Disadvantage	Low yield
Interesting fact	HEK-293 is the predominant cell line for transient expression of recombinant proteins.

Continues next page >

Methods of Protein Production Capture Sheet

ANSWER KEY **Do not share with students**

Continued

Station 3: Bacterial Cells

High-throughput recombinant protein expression in Escherichia coli: current status and future perspectives | Open Biology

Cell Type	Bacterial Cells
Prokaryotic or Eukaryotic	Prokaryotic
Description *What is the full name? What is it used for?*	Used to express and purify a specific protein
Advantage	Process is straightforward, fast cell growth/replication
Disadvantage	Incorrect protein folding; lacking certain enzymes necessary for certain protein components; low solubility of mammalian proteins; time intensive
Interesting fact	*Escherichia coli* is one of the most widely used microorganism species for producing recombinant proteins.

Station 4: Yeast Cells

Recombinant protein production in yeasts

Cell Type	Yeast Cells
Prokaryotic or Eukaryotic	Eukaryotic
Description *What is the full name? What is it used for?*	Said to express and purify a specific protein
Advantage	Single cell so has rapid cell growth and large protein yields; correct protein folding
Disadvantage	Produces high mannose residues which may make protein less efficient or cause immune response in humans
Interesting fact	Saccharomyces cerevisiae, Pichia pastoris, and Hansenula polymorpha are the most commonly used yeast cells.

Continues next page >

Methods of Protein Production Capture Sheet

ANSWER KEY **Do not share with students**

Continued

Station 5: Plant Cells

Critical Analysis of the Commercial Potential of Plants for the Production of Recombinant Proteins

Cell Type	Plant Cells
Prokaryotic or Eukaryotic	Eukaryotic
Description *What is the full name? What is it used for?*	Used to express and purify a specific protein
Advantage	Low cost
Disadvantage	Glycan structures of proteins are slightly different than what would be found in humans; produce a molecule that may be immunogenic (cause an immune response); low yields; inconsistent product quality; difficulty in large-scale production
Interesting fact	*Barley has been used to produce the human epidermal growth factor that is used as a cosmetic additive.*

Station 6: Recombinant Proteins Used in Humans Statistics

Recombinant pharmaceuticals from microbial cells: a 2015 update

According to Figure 1, which type of protein production has been approved more often?	Mammalian
Explain why you believe there are more approvals from the type chosen in #1.	The graph shows the approval for human drugs and because humans are mammals, it would make sense that the protein production type also is in mammals.
Use Figure 2 to list the order of the development of new drugs.	Discovery pre-clinical phase 1 phase 2 phase 3 pre-registration
According to Figure 3, what are the top four therapeutic areas that use marketed recombinant proteins? Read the chart carefully. You may also need to read the passages above the table.	Metabolic disorders (e.g., diabetes, obesity, or hypoglycaemia), others (cardiology, central nervous system, ophthalmology, and dermatology among others), haematological and oncology (e.g., renal anaemia, haemophilia A, bleeding, or clotting disorders).

Techniques in Protein Production Review

ANSWER KEY **Do not share with students**

Match each of the protein production methods (CHO, HEK-293, bacterial, yeast, plants) with the clue for which it belongs. More than one protein technique can be used for each statement.

1. I use eukaryotic cells.

CHO, HEK-293, Yeast, Plant

2. I can produce a protein yield of 10 g/L.

CHO

3. I am useful for proteins that are specific for the human body.

HEK-293

4. I should be used when trying to produce a protein that has several post-translational modifications such as phosphorylation or glycosylation.

HEK-293

5. I should be used if you need to save time.

yeast or bacterial cells, depending on other characteristics of the desired protein

6. I have the possibility of producing proteins that are immunogenic to their host.

plant and yeast cells

7. Using your notes from the scavenger hunt in the previous class session, create your own clue. Use clues #1-7 as a format to model your statement. Include your answer to the clue.

Answers will vary.

Small Group Alternative: Sorting Cards

ANSWER KEY **Do not share with students**

Directions

Cut out each box and rearrange them to form the correct order of events.

CHO Cells (2)	Eukaryotic cells	Produces a large yield of protein, up to 10 g/L	
HEK-293 Cells (3)	Eukaryotic cells	Useful for proteins that are specific for the human body	Used when trying to produce a protein that has several post-translational modifications such as phosphorylation or glycosylation
Bacterial Cells (1)	Should be used if you need to save time		
Yeast Cells (3)	Eukaryotic cells	Should be used if you need to save time	Could possibly produce proteins that are immunogenic to their host
Plant Cells (2)	Eukaryotic cells	Could possibly produce proteins that are immunogenic to their host	

Protein Purification Techniques Video Capture Sheet

ANSWER KEY **Do not share with students**

Directions
Answer the questions as your class watches the video: Methods for Protein Purification.

1. Identify reasons to purify a protein.

To make food, medicine, or to make materials

2. Why do proteins need to be purified?

Proteins are located in the cell and are mixed around with other molecules.

3. What are the four macromolecules that make up the cell? Put a circle around the one that we are discussing today.

(Protein)
Carbohydrates
Nucleic Acids
Lipids

4. What kinds of proteins can your body make?

Enzymes, antibodies

5. When you eat food with protein, what else are you also eating?

Lipid,
DNA,
RNA,
polysaccharide

6. Which technique can you use to separate proteins?

SDS-Page
Detergent and heat to break the cell open
Gel electrophoresis: separates protein by size
Mix with dye so DNA is visible

7. What characteristics of proteins can be used to help separate them from the cell?

negative charge, positive charge, size

Protein Purification Lab Tools and Protein Tags Capture Sheet

Part 2

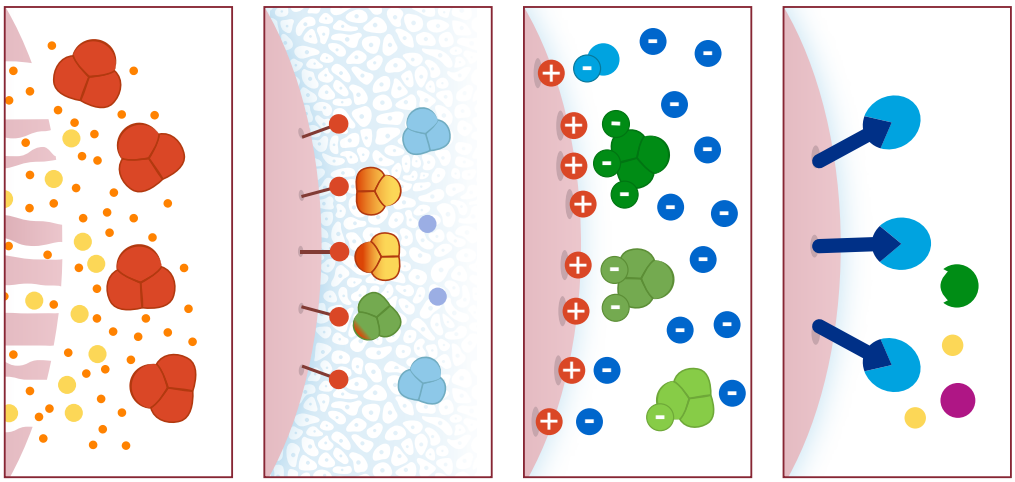
ANSWER KEY **Do not share with students**

Directions

Apply your knowledge from Part 1 by completing the questions below.

1a. Label the image with the correct protein purification method: ion exchange, affinity chromatography, gel filtration, hydrophobic interaction.

Protein Purification Methods



Gel filtration	Hydrophobic	Ion exchange	Affinity

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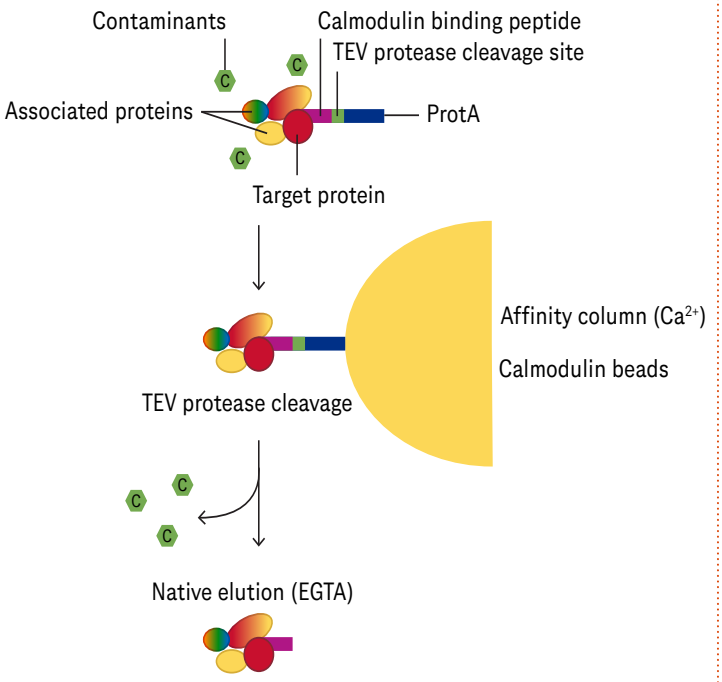
Protein Purification Lab Tools and Protein Tags Capture Sheet

Part 2

ANSWER KEY **Do not share with students**

Continued

Schematic Representation of Purification



2a. Protein Tag:

another example of affinity chromatography; not as specific as an antibody

2b. Goal

To purify a protein based on its affinity to a "bead" or recognition of a specific protein sequence

2c. Description of Process:

1.

The green contaminants need to be removed

2.

The affinity column is made of protein tags known as calmodulin beads

3.

Calmodulin beads recognize proteins that have a calmodulin binding site such as proteins belonging to the families of ribosomal proteins, proteasome and deubiquitinating enzymes.

Continues next page >

Protein Purification Lab Tools and Protein Tags Capture Sheet

Part 2

ANSWER KEY **Do not share with students**

Continued

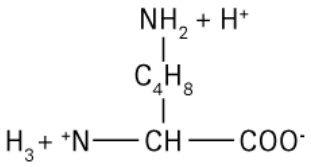
3. Complete the table using the information located *here*.

Protein Tag	CBP	His-Tag
Description *Full Name*	Calmodulin Binding Protein	Polyhistidine
Typical Use	Purification and Expression	Detection, Purification and Immobilization
Two characteristics *type of cell protein production systems it can be used in, can not be used in*	no endogenous *E. coli* proteins that bind calmodulin; not useful for purification from eukaryotic cells	Most common purification tag; short, linear recognition motif; one-step purification of 20%–80% pure protein

Protein Purification Review

ANSWER KEY — **Do not share with students**

Directions
Determine the most effective method of protein purification for each of the following characteristics of proteins. Include a rationale for your choice.

Protein Characteristics	**Hydrophobic, made of many C-H bonds**	 Lysine	**The MW of the protein is between 12,000–13,000 and contains a calmodulin binding domain**
SDS-Page	Used to verify presence of one purified protein	Used to verify presence of one purified protein	Used to verify presence of one purified protein
Affinity Chromatography			
Hydrophobic Interaction	This technique separates proteins based on their hydrophobicity		
Gel Filtration			This technique separates proteins based on the size
Ion Exchange		This technique separates proteins based on their charge; lysine is positively charged	
Protein Tag			Use of CBP tag can help isolate proteins associated with calmodulin

Continues next page >

Protein Purification Review

ANSWER KEY **Do not share with students**

Continued

Protein Characteristics	**Soluble in water and negatively charged**	**Antibodies have been produced for this protein**
SDS-Page	Used to verify presence of one purified protein	Used to verify presence of one purified protein
Affinity Chromatography		This technique uses antibodies that bind/recognize the protein
Hydrophobic Interaction		
Gel Filtration		
Ion Exchange	This technique separates proteins based on their charge. This protein is negatively charged. Since it is not hydrophobic, the hydrophobic interaction method would not be used	
Protein Tag		

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Diabetes and Insulin Tabletop Jigsaw Activator

Digital Access

Directions

Use the link provided to answer the question(s) that follow. Work with your tabletop group to agree on the best answer. Be prepared to share your answer with the class.

Group 1

Diabetes: Types, Risk Factors, Symptoms, Tests, Treatments & Prevention

What is diabetes?

Group 2

A Snapshot: Diabetes In The United States | Diabetes

Use the infographic to answer the following question:

Why is diabetes a problem in the United States?

Group 3

A Snapshot: Diabetes In The United States | Diabetes

Diabetes: Types, Risk Factors, Symptoms, Tests, Treatments & Prevention

What is the difference between Type 1 and Type 2 diabetes?

Group 4

US Diabetes Surveillance System

What has happened to the number of individuals in the United States with diabetes in the last 18 years?

Click on your state.

What percent of the population in your state have been diagnosed with diabetes?

How does this compare to the national level?

Group 5

A Snapshot: Diabetes In The United States | Diabetes

What is the best way to treat or prevent diabetes?

Group 6

Diabetes: Types, Risk Factors, Symptoms, Tests, Treatments & Prevention

What is insulin?

Group 7

Insulin and Diabetes Management Insulin

Where did the first commercially produced insulin come from?

What happened in the 1980s?

Group 8

Insulin and Diabetes Management Diabetes Remedies Before Insulin

What was the most effective treatment for diabetes before insulin?

FUTURELAB+

Diabetes and Insulin Tabletop Jigsaw Activator

Print Access

Directions

Use the QR code provided to answer the question(s) that follow. Work with your tabletop group to agree on the best answer. Be prepared to share your answer with the class.

Group 1 What is Diabetes?		Group 5 What is the best way to treat or prevent diabetes?	
Group 2 Why is diabetes a problem in the US?		Group 6 What is insulin?	
Group 3 What is the difference between Type 1 and Type 2 diabetes?		Group 7 Where did the first commercially produced insulin come from? What happened in the 1980s?	
Group 4 What has happened to the number of individuals in the US with diabetes in the last 18 years?		Group 8 What was the most effective treatment for diabetes before insulin?	

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Diabetes and Insulin Tabletop Jigsaw Capture Sheet

Directions
Take notes on your assigned topic. Work with your tabletop group to agree on the best answer to the activator question(s). Take notes on the remaining topics as others share their learning.

My Topic:	
My Notes:	

Group 1 Diabetes Overview		Group 5 Diabetes Treatment or Prevention	
Group 2 Diabetes In The United States		Group 6 Insulin Information	
Group 3 Type 1 and Type 2 Diabetes		Group 7 Insulin and Diabetes Management	
Group 4 US Diabetes Surveillance System		Group 8 Diabetes Remedies Before Insulin	

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Methods of Protein Production Station Links

Directions
Use the information located at each station to complete the chart. You will have six minutes at each station. Use the first two resources to find descriptions of each method. Choose which group member will locate specific information from the chart.

All Stations			
Station 1: CHO Cells		**Station 4: Yeast Cells**	
Station 2: HEK-293 Cells		**Station 5: Plant Cells**	
Station 3: Bacterial Cells		**Station 6: Recombinant Proteins Used in Humans Statistics**	

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Methods of Protein Production Capture Sheet

Directions
Use the information located at each station to complete the chart. You will have six minutes at each station. Use the first two resources to find descriptions of each method. Choose which group member will locate specific information from the chart.

Station 1: CHO Cells

CHO cells in biotechnology for production of recombinant proteins: current state and further potential

CHO cells can make more protein

Cell Type	CHO Cells
Prokaryotic or Eukaryotic	
Description *What is the full name? What is it used for?*	
Advantage	
Disadvantage	

Station 2: HEK-293 Cells

A Multi-Omics Analysis of Recombinant Protein Production in HEK-293 Cells

Cell Type	HEK-293 Cells
Prokaryotic or Eukaryotic	
Description *What is the full name?*	
Advantage	
Disadvantage	

Continues next page >

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Methods of Protein Production Capture Sheet

Continued

Station 3: Bacterial Cells

High-throughput recombinant protein expression in Escherichia coli: current status and future perspectives | Open Biology

Cell Type	Bacterial Cells
Prokaryotic or Eukaryotic	
Description *What is the full name? What is it used for?*	
Advantage	
Disadvantage	

Station 4: Yeast Cells

Recombinant protein production in yeasts

Cell Type	Yeast Cells
Prokaryotic or Eukaryotic	
Description *What is the full name? What is it used for?*	
Advantage	
Disadvantage	

Continues next page >

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Methods of Protein Production Capture Sheet

Continued

Station 5: Plant Cells

Critical Analysis of the Commercial Potential of Plants for the Production of Recombinant Proteins

Cell Type	Plant Cells
Prokaryotic or Eukaryotic	
Description *What is the full name? What is it used for?*	
Advantage	
Disadvantage	

Station 6: Recombinant Proteins Used in Humans Statistics

Recombinant pharmaceuticals from microbial cells: a 2015 update

According to Figure 1, which type of protein production has been approved more often?	
Explain why you believe there are more approvals from the type chosen in #1.	
Use Figure 2 to list the order of the development of new drugs.	
According to Figure 3, what are the top four therapeutic areas that use marketed recombinant proteins? Read the chart carefully. You may also need to read the passages above the table.	

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Techniques in Protein Production Review

Directions
Match each of the protein production methods (CHO, HEK-293, bacterial, yeast, plants) with the clue for which it belongs. More than one protein technique can be used for each statement.

1. I use eukaryotic cells.

_____

2. I can produce a protein yield of 10 g/L.

_____

3. I am useful for proteins that are specific for the human body.

_____

4. I should be used when trying to produce a protein that has several post-translational modifications such as phosphorylation or glycosylation.

_____

5. I should be used if you need to save time.

_____

6. I have the possibility of producing proteins that are immunogenic to their host.

_____

7. Using your notes from the scavenger hunt yesterday, create your own clue. Use #1–7 as a format to model your statement. Include your answer to the clue.

_____

_____

_____

_____

_____

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Small Group Alternative: Sorting Cards

1 of 2 pages

Directions

Cut out each box and rearrange them to form the correct order of events.

CHO Cells (2)	Eukaryotic cells
HEK-293 Cells (3)	Eukaryotic cells
Yeast Cells (3)	Eukaryotic cells
Plant Cells (2)	Eukaryotic cells
Bacterial Cells (1)	Produces a large yield of protein, up to 10 g/L

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Small Group Alternative: Sorting Cards

2 of 2 pages

Directions

Place the Stages of Vaccine Production cards in the correct order of events.

Used when trying to produce a protein that has several post-translational modifications such as phosphorylation or glycosylation	Useful for proteins that are specific for the human body
Should be used if you need to save time	Could possibly produce proteins that are immunogenic to their host
Should be used if you need to save time	Could possibly produce proteins that are immunogenic to their host

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Protein Purification Techniques Video Capture Sheet

Directions
Answer the questions as your class watches the video: Methods for Protein Purification.

1. Identify reasons to purify a protein.

2. Why do proteins need to be purified?

3. What are the four macromolecules that make up the cell? Put a circle around the one that we are discussing today.

4. What kinds of proteins can your body make?

5. When you eat food with protein, what else are you also eating?

6. Which technique can you use to separate proteins?

7. What characteristics of proteins can be used to help separate them from the cell?

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Protein Purification Lab Tools and Protein Tags Capture Sheet

Part 1

Directions

Complete the table as your class discusses different techniques of protein purification.

Lab Tool	Rationale For Its Use
SDS-Page	
Affinity Chromatography	
Hydrophobic Interaction	
Gel Filtration	
Ion Exchange	

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Protein Purification Lab Tools and Protein Tags Capture Sheet

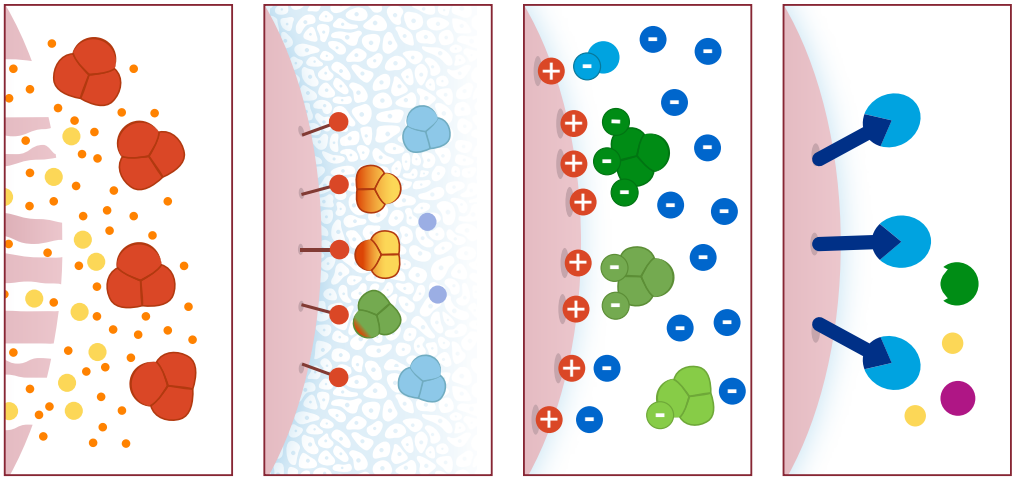
Part 2

Directions

Apply your knowledge from Part 1 by completing the questions below.

1a. Label the image with the correct protein purification method:
ion exchange, affinity chromatography, gel filtration, hydrophobic interaction.

Protein Purification Methods



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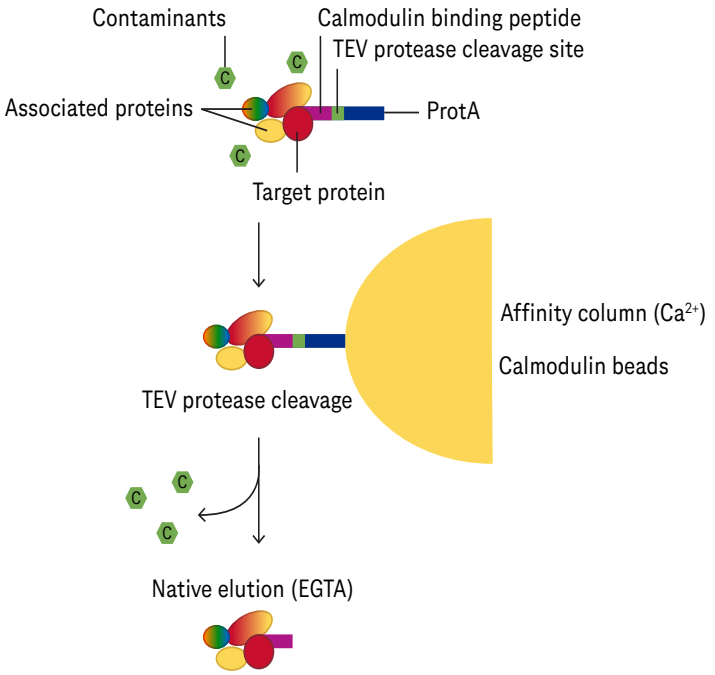
Protein Purification Lab Tools and Protein Tags Capture Sheet

Part 2

Continued

Schematic Representation of Purification



2a. Protein Tag:

2b. Goal

2c. Description of Process:

1.

2.

3.

Continues next page >

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Protein Purification Lab Tools and Protein Tags Capture Sheet

Part 2

Continued

3. Complete the table using the information located *here*.

Protein Tag	**CBP**	**His-Tag**
Description *Full Name*		
Typical Use		
Two characteristics *type of cell protein production systems it can be used in, cannot be used in*		

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Protein Purification Flowchart Assignment

Directions

You will be tasked with determining the most effective methods to purify a specific protein with certain characteristics. This protein is included in a mixture of other proteins with various (some similar) characteristics. You must select two to three different purification methods. Show your purification process as a flowchart. Find an example here.

Name of Protein Purification Method:

Step 1	Short Description	Rationale	Result
↓			
Step 2	Short Description	Rationale	Result
↓			
Step 3	Short Description	Rationale	Result
↓			
Step 4	Short Description	Rationale	Result

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Protein Purification Flowchart Rubric

Score	4	3	2	1
Organization	The flowchart is clear and organized, including sequential steps.	The flowchart is organized, including sequential steps.	The flowchart is unorganized and steps are clearly skipped.	The flowchart is unorganized and steps are unclear and missing.
Description	Steps used to purify protein make sense and are realistic to protein characteristics.	Steps used to purify protein make sense, but are unrealistic due to protein characteristics.	Steps used to purify protein make some sense according to protein characteristics.	Steps used to purify protein do not make sense and are unrealistic.
Results	Includes at least two sentences with supporting evidence such as an image	Includes at least one sentence with supporting evidence such as an image	Includes two sentences, but no supporting evidence	Includes one sentence but no supporting evidence or rationale
Final Score				

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Protein Purification Lab Manual Assignment

Directions

Your group has been tasked with creating a lab manual for student lab trainees to use during a summer science program. The lab manual must include descriptions of protein purification techniques and the required lab materials. This will ultimately assist with helping student lab trainees with understanding these various techniques including

1. *gel filtration*
2. *ion exchange chromatography*
3. *hydrophobic interaction chromatography or*
4. *affinity chromatography*

The lab manual must include:

1. Table of Contents
2. Description of the process (one per group member) and appropriate vocabulary terms
   a. Section for the purpose
   b. Section for the lab material required (at least three items excluding the target protein)
   c. Step-by-step process (how does it work?)
   d. Appropriate use of vocabulary terms
3. Pictures—one per process (text: image ratio = 2:1)
4. Model of each technique with labeled components
5. 30-second video explaining the model (insert link into lab manual)

Resources

Here is an overview of protein purification methods from Chapter 2 in this *handbook*.

Use the following page numbers to locate information about each topic.

1. Gel filtration, pg 23
2. Ion Exchange Chromatography, pg 25
3. Hydrophobic Interaction Chromatography, pg 27
4. Affinity Chromatography, pg 19

In addition, use the specific protein purification handbooks located *here* to help create your presentation.

Tools

The following tools can be used to build your model: cotton balls, beads, cotton swabs, empty toilet tissue and paper towel rolls, tissue, aluminum foil, modeling clay, toothpicks, tape, scissors, puff balls, pipe cleaners, paper stars, craft stick, rubberband

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Protein Purification Lab Manual Rubric

Score	4	3	2	1
Model	The model is labeled and includes the essential portion of the protein purification method.	The model is labeled and includes most of the essential portions of the protein purification method.	The model is not labeled and/or missing most of the essential portions of the protein purification method.	The model is not labeled and is missing all of the essential portions of the protein purification method.
Description of Process	Description is specific, clear, and the procedure is mentioned in an organized and step-by-step fashion and includes necessary lab material.	Description is clear and the procedure is organized and includes necessary lab material, but missing specific method-associated vocabulary terms.	Description is organized but the procedure is unorganized or is missing necessary lab material or specific method-associated vocabulary terms.	Description is not organized and missing specific method-associated vocabulary terms.
Video Recording	The recording was clear and to the point. Any relevant vocabulary terms were mentioned and explained.	The recording was clear and to the point. Relevant vocabulary was mentioned, but not explained.	The recording was not to the point. Vocabulary was missing.	The recording was unclear.
Final Score				

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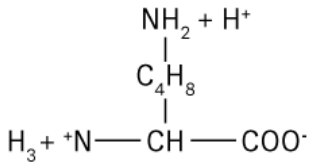
Protein Purification Review

Directions
Determine the most effective method of protein purification for each of the following characteristics of proteins. Include a rationale for your choice.

Protein Characteristics	**Hydrophobic, made of many C-H bonds**	 Lysine
SDS-Page		
Affinity Chromatography		
Hydrophobic Interaction		
Gel Filtration		
Ion Exchange		
Protein Tag		

Continues next page >

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Protein Purification Review

Continued

Protein Characteristics	**The MW of the protein is between 12,000–13,000**	**Soluble in water and negatively charged**
SDS-Page		
Affinity Chromatography		
Hydrophobic Interaction		
Gel Filtration		
Ion Exchange		
Protein Tag		

Continues next page >

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Protein Purification Review

Continued

Protein Characteristics	**Antibodies have been produced for this protein.**
SDS-Page	
Affinity Chromatography	
Hydrophobic Interaction	
Gel Filtration	
Ion Exchange	
Protein Tag	

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Nonhuman Proteins Used in Humans Assignment

Directions

You will play the role of a biochemist and investigate an example of a protein that can be purified and used to treat humans. Examples of some recombinant proteins used in clinical medicine include:

Coagulation factors	*Human Growth Hormone*
Insulin	*Tissue Plasminogen Activator*
Glucocerebrosidase	*DNase I*
Erythropoietin	*Alpha Interferon*

The following components will be included in your final paper:

1. Name of protein
2. Description of therapeutic use
3. Choice of protein production technique and reason why this is the source
4. Flowchart of process used to purify protein (including rationale for each step)
5. Two social justice challenges with using proteins as therapeutics
6. At least two references

Resources

Besides the articles below, a Google search can be performed using the key words, "Protein Name" + "clinical medicine" or "Protein name" + "Recombinant protein." These searches should help with locating additional sources; be sure to make sure they are reputable sources.

Recent Developments in Bioprocessing of Recombinant Proteins: Expression Hosts and Process Development

Recombinant Protein Therapeutics | List of High Impact Articles | PPts | Journals | Videos

Production of Recombinant Pharmaceutical Proteins

The 5 Most Pressing Ethical Issues in Biotech Medicine

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Nonhuman Proteins Used in Humans Rubric

Score	4	3	2	1
Rationales	The rationales provided are clearly stated and include supporting evidence.	The rationales provided include supporting evidence.	The rationales provided include little supporting evidence.	The rationales provided are not clearly stated and do not include supporting evidence.
Flowchart of Process	The flowchart is organized and includes succinct statements.	The flowchart is organized and includes long explanation statements.	The flowchart is not very organized and includes incomplete statements.	The flowchart is unorganized and statements are missing.
Social Justice Challenge	At least two social justice challenges are identified and relevant to the use of the protein.	One social justice challenge is identified relevant to the use of the protein.	One social justice challenge is identified, but is unclear or not relevant to the use of the protein.	No social justice challenges are identified.
Final Score				

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References

Laura Sanchez-Garcia, Lucas Martin, Ramon Mangues, Neus Ferrer-Miralles, Esther Vazquez and Antonio Villaverde. *Recombinant pharmaceuticals from microbial cells: a 2015 update*. *Springer Link, Microbial Cell Factories. 2016.*

Nabih Baeshen, Mohammed Baeshen, Abdullah Sheikh, Roop Bora, Mohamed Ahmed, Hassan Ramadan, Kulvinder Saini, Elrashdy Redwan. *Cell factories for insulin production.* *Microbial Cell Factories, 2014.*

The Tandem Affinity Purification (TAP) Method: A General Procedure of Protein Complex Purification. *Methods 24, no. 3(2001): 218-29.*

Young, Carissa L., Zachary T. Britton and Anne S. Robinson. *Recombinant protein expression and purification: a comprehensive review of affinity tags and microbial applications.* Biotechnology Journal 7, no.5 (2012): 620-634.

Strategies for Protein Purification. GE Healthcare.

Unintended Consequences and Hidden Obstacles in Medicine Access in Sub-Saharan Africa. (2019). PubMed Central (PMC).