

Protein purification for GFP

Focus questions	How can proteins be purified? What is the mechanism used in biotechnology to extract proteins of interest?
Learning target	Students remove a colony of transformed bacteria that results from the pGLO™ lab and treat it to remove and purify the green fluorescent protein (GFP) produced by the gene.
Vocabulary	Hydrophobic interaction column chromatography, lyse, supernatant, hydrophobic, hydrophilic

This lesson uses Bio-Rad pGLO™ Bacterial Transformation Kit, Catalog #166-0005EDU available from explorer.bio-rad.com.

ETS1.C Optimizing the Design Solution

Performance expectation HS-LS1-1	Classroom connection: Students are given the challenge to remove a protein from bacteria that produced it.
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Science and engineering practices

Constructing Explanations and Designing Solutions	Classroom connection: The kit comes with specific instructions for how to purify and remove the protein. Students experiment with the buffers to determine the order in which to use each to purify the protein.
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Disciplinary core ideas

ETS1-C. Optimizing the Design Solution	Classroom connection: Students research to determine the best way to extract and purify the protein.
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Cross-cutting concepts

Structure and Function	Classroom connection: Students will use the known structures of bacteria and the known structures of GFP to determine how to break down the bacteria to remove the protein and purify it through hydrophobic interaction column (HIC) chromatography.
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Background

Protein purification uses a variety of methods to isolate a protein from cellular products. Common examples include isolating lactase, soy protein or insulin for commercial or medicinal use. Different methods are used based on the chemical and physical properties of the protein of interest.

This activity utilizes hydrophobic interaction column (HIC) chromatography to isolate GFP from transformed bacteria (see Biotechnology Lesson 6, Part 2).

The lesson is divided into two potential options for teaching students:

- **Option 1:** Students begin by using one colony of transformed bacteria from each condition in the pGLO™ transformation lab. The colonies are added to nutrients to grow, then incubated. After 24-48 hours, the bacteria is centrifuged, resuspended, then an enzyme is added to **lyse** the bacterial membranes. The samples are frozen to complete the breakdown of the bacteria, then centrifuged again to remove the bacterial debris. The remaining protein samples are then added to the chromatography columns which contain a “bed” of microbeads, and these columns are treated with buffers of high salt concentrations that retain the hydrophobic (water-hating) protein (GFP). The final treatment rinses the protein from the column. Students can be given the instructions to follow and obtain the results. This is the traditional method of using the lab, wherein students follow the instructions.
- **Option 2:** Students are given a challenge to determine how to isolate and purify the GFP protein produced by the bacteria in the previous lab. Students must research to determine how to break down the bacteria, then how to purify it using the columns and materials provided. The instructions provided still have the steps for breaking down the bacteria and purifying the protein, but have the chemical and buffer names removed. If desired, no instructions might be given except the challenge. The first page of the student handout for Option 2 does not reference the instructions.

Prior knowledge

Students need to have completed the BioRad pGLO™ Lab and have bacteria available from each of the conditions tested in that lab. Students who complete this lab should have advanced lab skills to be able to follow the multiple steps over multiple days of this lab. In addition, the combination of salts used are specifically sequenced to remove the protein and purify it. A good understanding of hydrophobic and hydrophilic properties is helpful.

Timeline

See page 4 of the lab manual: bio-rad.com/webroot/web/pdf/lse/literature/4006099.pdf

- **Lesson 1:** Introduction to purification
- **Lesson 2:** Picking colonies and inoculating cell cultures
- **Lesson 3:** Purification phase 1: bacterial concentration and lysis
- **Lesson 4:** Purification phase 2: removing bacterial debris
- **Lesson 5:** Purification phase 3: protein chromatography

Each lesson takes a portion of the class period over 5 days.

Materials

- Hot plate with magnetic stir option
- Magnetic stir bar
- 250 mL Flask
- 55 mL distilled water
- LB tablet (included in kit)
- TE buffer (included in kit)
- Arabinose (included in kit)
- Ampicillin (included in kit)
- UV lights (included in kit)
- Sterile loops (included in kit)
- Sterile pipettes (included in kit)
- Test tube racks
- Test tubes
- Cups
- Rocking table or shaking incubator or incubator (set at 32° C)
- Centrifuge

Teacher preparation

Lesson 1

Review of the principles used in the pGLO™ transformation lab. This includes a discussion of growth media, antibiotic selection, and gene regulation (see pages 9–10 of the lab manual). At this point it is recommended to describe the process or ask students to research the steps that will be used to purify the protein and each can be reviewed as the step is completed. Divide students into the groups they were in for the pGLO™ lab.

Lesson 2

Choosing colonies and inoculating cell cultures (see pages 6–8 in the lab manual). Rehydrate the ampicillin and arabinose using TE buffer. Prepare the liquid media by heating distilled water and dissolving the LB tablet in the water.

- Again, the recommendation is to use a hot plate and stir bar, even though the manual suggests using a microwave.
- Allow the media to cool to below 55° C.
- Add 0.5 mL of arabinose and 0.5 mL of ampicillin to the flask of LB media.
- Mix by swirling.
- Aliquot 2 mL of the culture media into the 2 culture tubes per station. (Store culture tubes in refrigerator until ready to use.)

Students inoculate the tubes with a colony from their pGLO™ lab. Provide sterile loops and pipettes. The colonies should be shaken during incubation if possible. If not, students need to vigorously shake the culture tubes for at least 30 seconds before placing in the incubator set at 32° C on their sides. (*Be sure lids are sealed.*)

Lesson 3: Purification phase 1 (see page 8 in the lab manual)

- Rehydrate the vial of lyophilized lysozyme with TE buffer. Mix gently to aid in the resuspension. Keep the vial of lysozyme on ice or in a refrigerator until use.
- Set up lab stations with a microtube containing 1 mL of TE solution and pipettes for each group.
- Multiple centrifuges may be needed as students need to use for 5 minutes at max speed.
- Students will use 1 drop of lysozyme.
- Tubes will be stored in the freezer overnight.

Lesson 4: Purification phase 2 (see page 8 in the lab manual)

- Set up lab stations with pipettes, chromatography columns, equilibration buffer, and binding buffer for each group.
- Students will prepare chromatography columns as they centrifuge their samples.
- Students centrifuge lysed cells for 10 minutes.

Lesson 5: Purification phase 3 (see page 8 in the lab manual)

Set up lab stations with test tubes, previously prepared chromatography tubes, pipettes, wash buffer, and TE buffer. Students load their lysed cells into the chromatography columns and run the protein through them.

Differentiation

Other ways to connect with students with various needs:

- **Local community:** Students may visit a local biotech lab or look for a virtual tour of a biotech lab where proteins are extracted and purified.
- **Students with special needs (language/reading/auditory/visual):** The directions for the lab are both visual and written. Students who need additional support may watch GFP Purification at youtu.be/H7wv1hpfc_M (Skip the Edvotek portion of the video from 1:18-3:47, unless you want them to run this protocol without using the GFP, but only food coloring/dye.)
- **Extra support:** If students are struggling, they may watch Protein Purification GFP at youtu.be/H7wv1hpfc_M. The narrator is a fast talker, but the overview is good.
- **Extensions:** Students may complete Option 2 with support of the directions or without.

Assessments

Rubric for assessment

Skill	Developing	Satisfactory	Exemplary
Design a solution to a complex real-world problem by breaking it down into smaller, more manageable problems that can be solved through engineering.	Student was unable to extract and/or purify GFP.	Student could break the process of GFP purification into smaller steps; extracting GFP from the bacteria, then purifying GFP using HIC chromatography.	Student researched and successfully carried out the procedure of extracting GFP from bacteria based on the physical properties of bacterial membranes.
Prediction	Student incorrectly predicted the outcome of the buffers.	Student correctly predicted the outcome of using each of the buffers in the columns.	Student determined the proper buffers to use in the proper order to purify GFP using the properties of GFP.
			Student successfully carried out the procedure to purify and collect GFP.

Rubric for self-assessment

Skill	Yes	No	Unsure
My group's predictions about the effects of each of the buffers were correct.			
I am able to explain the function/role of each buffer in the process of chromatography.			
We were successful in isolating and purifying GFP from the bacterial cells.			