

Lab 9 - Green Fluorescent Protein (GFP) Purification Practice Test (Sample)

Study Guide



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SAMPLE

Questions

SAMPLE

- 1. What type of buffer is typically used during the initial binding step?**
 - A. Elution Buffer**
 - B. Binding Buffer**
 - C. Equilibration Buffer**
 - D. Wash Buffer**
- 2. What is important to ensure when draining the chromatography column?**
 - A. It drains quickly**
 - B. All liquid buffer drains out completely**
 - C. It remains capped during drainage**
 - D. Temperature is maintained**
- 3. What is the function of the centrifuge in the bacterial concentration exercise?**
 - A. To mix the solutions thoroughly**
 - B. To heat the samples**
 - C. To pellet the bacteria and separate it from the growth media**
 - D. To freeze the samples quickly**
- 4. Which buffer is used to cause GFP to unbind from the column?**
 - A. Equilibration Buffer**
 - B. Binding Buffer**
 - C. Elution Buffer**
 - D. TE (Elution) Buffer**
- 5. When transferring supernatant into a new microcentrifuge tube, what is the suggested volume?**
 - A. 150 uL**
 - B. 200 uL**
 - C. 250 uL**
 - D. 300 uL**

- 6. What does a bacterial colony represent?**
- A. A mix of different bacterial cells**
 - B. A large cluster of bacterial cells from a single clonal cell**
 - C. Individual bacterial cells in a liquid culture**
 - D. A single type of antibiotic-resistant bacterium**
- 7. What is the total volume of culture transferred to the microcentrifuge tube in Exercise 1?**
- A. 1mL**
 - B. 2mL**
 - C. 0.5mL**
 - D. 3mL**
- 8. How many collection tubes should be obtained and labeled for the chromatography step?**
- A. 1**
 - B. 2**
 - C. 3**
 - D. 4**
- 9. How can a cancer-curing protein be recovered from bacterial cells?**
- A. By freezing and thawing the cells**
 - B. By isolating a colony and lysing the cells**
 - C. By filtering through a membrane**
 - D. By subjecting the cells to high temperatures**
- 10. What happens to the proteins as they pass through the chromatography column?**
- A. They evaporate.**
 - B. They adhere to the beads.**
 - C. They move downward through spaces between beads.**
 - D. They increase in temperature.**

Answers

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1. B
2. B
3. C
4. D
5. C
6. B
7. B
8. C
9. B
10. C

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Explanations

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1. What type of buffer is typically used during the initial binding step?

- A. Elution Buffer**
- B. Binding Buffer**
- C. Equilibration Buffer**
- D. Wash Buffer**

The initial binding step in protein purification, particularly for affinity chromatography used in isolating proteins like Green Fluorescent Protein (GFP), typically employs a binding buffer. This buffer is specifically formulated to create optimal conditions for the targeted protein to adhere to the chromatography medium. The binding buffer usually maintains a neutral pH and salt concentration that promotes the correct folding and charge characteristics of the protein, allowing it to effectively bind to the corresponding ligand or matrix present in the chromatography system. By ensuring these conditions, the binding buffer plays a crucial role in maximizing the yield of the purified protein. In contrast, other buffers mentioned have different purposes. The elution buffer is designed to release the bound protein from the column, the equilibration buffer prepares the chromatography medium before samples are introduced, and the wash buffer is used to rinse away any unbound or nonspecifically bound proteins after the initial binding step. Each of these serves a distinct role in the overall purification process, but does not serve the specific purpose of enhancing the binding of proteins, which is why the binding buffer is essential at this stage.

2. What is important to ensure when draining the chromatography column?

- A. It drains quickly**
- B. All liquid buffer drains out completely**
- C. It remains capped during drainage**
- D. Temperature is maintained**

When draining the chromatography column, it is crucial to ensure that all liquid buffer drains out completely. This is important because any remaining liquid can dilute the sample that is later introduced and can also affect the binding and elution properties of the stationary phase within the column. If not completely drained, residual buffer may alter the concentration of the fractions collected, leading to inconsistency in results and compromising the purification process of green fluorescent protein (GFP). Furthermore, draining the column fully helps to minimize contamination and ensures that any bound proteins or compounds interact appropriately with the washing or elution buffers that follow. This step is essential for achieving high purity levels and optimal yields of the target protein, which is critical for successful future applications or analyses.

3. What is the function of the centrifuge in the bacterial concentration exercise?

- A. To mix the solutions thoroughly**
- B. To heat the samples**
- C. To pellet the bacteria and separate it from the growth media**
- D. To freeze the samples quickly**

The function of the centrifuge in the bacterial concentration exercise is to pellet the bacteria and separate it from the growth media. In this process, the centrifuge spins samples at high speeds, creating a strong centrifugal force that causes denser materials, like bacterial cells, to move to the bottom of the tube, forming a pellet. This allows for the effective separation of the bacteria from the liquid growth media, which contains nutrients and other components that are not needed for further analysis of the bacterial cells. This function is critical in many molecular biology and biochemistry procedures, including the isolation of proteins like Green Fluorescent Protein (GFP), as it enables researchers to concentrate the cells for further processing, such as lysis and extraction of the desired proteins. In this particular context of GFP purification, efficient pelleting ensures that the maximum amount of bacterial cells can be processed, which is essential for obtaining a high yield of GFP.

4. Which buffer is used to cause GFP to unbind from the column?

- A. Equilibration Buffer**
- B. Binding Buffer**
- C. Elution Buffer**
- D. TE (Elution) Buffer**

The correct buffer used to cause GFP to unbind from the column is an elution buffer. Elution buffers are specifically designed to disrupt interactions between the target protein, in this case, GFP, and the matrix of the chromatography column. This disruption allows the protein to be released or "eluted" from the column into the collected fractions. Typically, elution buffers often contain higher concentrations of salt, different pH values, or specific ligands that induce changes in the binding interactions, enabling the target protein to detach effectively. In the context of GFP purification, the elution buffer chosen would have a composition that supports the stable release of GFP while ensuring that its structure and function are preserved. The other buffers listed serve different functions in the purification process: equilibration buffers prepare the column for binding by establishing the proper ionic conditions, and binding buffers provide the appropriate conditions to promote the attachment of GFP to the column. Thus, they do not facilitate the unbinding process necessary for elution.

5. When transferring supernatant into a new microcentrifuge tube, what is the suggested volume?

- A. 150 uL
- B. 200 uL
- C. 250 uL**
- D. 300 uL

The correct choice of 250 μ L for transferring supernatant into a new microcentrifuge tube is based on the typical volume ranges used in molecular biology protocols for handling supernatants. This volume allows for efficient processing while maximizing the yield of the target protein, in this case, green fluorescent protein (GFP), without exceeding the capacity of standard microcentrifuge tubes, which typically can hold up to 1.5 mL. Additionally, using 250 μ L strikes a balance between being a sufficient amount to isolate the protein while also permitting easy mixing or further processing in subsequent steps, such as purification or analysis. Choosing volumes that are too low, such as 150 μ L or 200 μ L, may not capture enough of the supernatant, potentially leading to lower yields of GFP. A volume as high as 300 μ L could risk the limitations of the microcentrifuge tube's capacity, which may lead to spills or inaccurate measurements if the tube overfills during centrifugation. Thus, 250 μ L is the most practical and effective choice for these procedures.

6. What does a bacterial colony represent?

- A. A mix of different bacterial cells
- B. A large cluster of bacterial cells from a single clonal cell**
- C. Individual bacterial cells in a liquid culture
- D. A single type of antibiotic-resistant bacterium

A bacterial colony represents a large cluster of bacterial cells that originate from a single clonal cell. This means that all the cells in the colony are genetically identical, having arisen from the division of one bacterium. When bacteria are cultured on a solid medium, each colony starts from a single original cell that divides and multiplies, forming a visible group. This characteristic makes colonies valuable in microbiology for isolating specific strains, studying their characteristics, and performing experiments. In contrast, a mix of different bacterial cells would not form a distinct colony since the cells would come from different origins and not be genetically identical. Individual bacterial cells in a liquid culture do not form colonies, as they are dispersed in the liquid medium. A single type of antibiotic-resistant bacterium does not encompass the broader nature of what a colony represents, as it might limit the definition to only one specific feature, rather than the general concept of clonal growth.

7. What is the total volume of culture transferred to the microcentrifuge tube in Exercise 1?

- A. 1mL
- B. 2mL**
- C. 0.5mL
- D. 3mL

In the context of the lab exercise, the total volume of culture transferred to the microcentrifuge tube would typically be dictated by the standard protocol used for GFP purification practices and the capacities of the equipment involved. In many experiments, especially those focusing on the expression and purification of proteins like GFP, the volume transferred often falls within a range that allows for comprehensive recovery and analysis of the proteins of interest. Choosing a total volume of 2 mL suggests that this amount is sufficient to yield a good concentration of the GFP for subsequent purification steps, while still being manageable to handle in a microcentrifuge tube. A volume of 1 mL might not capture the entirety of the volume needed for optimal recovery, while smaller volumes, like 0.5 mL, may not provide enough biomass for significant experimental outcomes. On the other end, a volume of 3 mL might be unnecessarily large, possibly complicating steps later in the purification process. Thus, 2 mL strikes a balance between adequate sample size and practical handling, making it the most logical choice in this context.

8. How many collection tubes should be obtained and labeled for the chromatography step?

- A. 1
- B. 2
- C. 3**
- D. 4

For the chromatography step in the purification of Green Fluorescent Protein (GFP), obtaining and labeling three collection tubes allows for effective fractionation and analysis of the eluted proteins. Typically, during the chromatography process, different fractions are collected as the sample passes through the chromatography column. Each fraction may contain different amounts of the target protein, as well as impurities or other components. By utilizing three collection tubes, you can adequately separate these fractions to identify which one contains the highest concentration of GFP. This approach not only aids in maximizing the yield of the desired protein but also provides opportunities for further analysis, such as determining the purity of the fractions collected. Proper labeling of the tubes ensures that you can track which fraction corresponds to each collection point, facilitating any necessary downstream applications, such as further purification steps, characterization, or analysis. In summary, the choice of three collection tubes supports an organized and systematic approach to protein purification, allowing for effective separation and evaluation of the eluted GFP.

9. How can a cancer-curing protein be recovered from bacterial cells?

- A. By freezing and thawing the cells**
- B. By isolating a colony and lysing the cells**
- C. By filtering through a membrane**
- D. By subjecting the cells to high temperatures**

The recovery of a cancer-curing protein from bacterial cells is effectively achieved by isolating a colony and lysing the cells. This process involves growing bacterial cultures that express the desired protein and then breaking open the cells to release the intracellular contents, including the target protein. Lysing the cells is crucial because it allows the proteins inside the bacterial cells to be extracted for further purification. This method can utilize various techniques, such as mechanical disruption, chemical lysis (using detergents), or enzymatic treatments, all of which are designed to ensure that the protein remains intact and functional for subsequent experiments or therapeutic applications. The other methods, while having their own utility, do not primarily focus on the efficient recovery of proteins from cells. Freezing and thawing can lead to cell disruption but may not be as effective for protein extraction, and filtering through a membrane is generally used for purification rather than initial recovery after cell lysis. Subjecting cells to high temperatures can denature proteins, rendering them non-functional, and is usually not a reliable method for recovering sensitive proteins like a cancer-curing protein.

10. What happens to the proteins as they pass through the chromatography column?

- A. They evaporate.**
- B. They adhere to the beads.**
- C. They move downward through spaces between beads.**
- D. They increase in temperature.**

As proteins pass through the chromatography column, they primarily move downward through the spaces between the beads. This movement is facilitated by the flow of the mobile phase, which carries the proteins along with it. The column is typically packed with beads that provide a stationary phase, which can interact with the proteins in various ways depending on the type of chromatography being used (such as size exclusion, affinity, or ion exchange). In size exclusion chromatography, for instance, smaller proteins enter the pores of the beads and are temporarily delayed, while larger proteins are excluded from these pores, allowing them to elute more quickly. In affinity chromatography, specific interactions between the proteins and the substances attached to the beads cause certain proteins to bind to the stationary phase while others pass through. However, in both cases, the primary action involves the proteins moving through the interstitial spaces in the column, demonstrating their differing affinities or sizes based on the designed application of the chromatography. Thus, this movement is a key aspect of the purification process, allowing for effective separation of proteins based on their physical or chemical properties.