

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

Study Guide



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SAMPLE

Questions

SAMPLE

- 1. What occurs to GFP when incubated in a high salt Binding Buffer?**
 - A. It precipitates out of solution**
 - B. It undergoes conformational change**
 - C. It remains completely soluble**
 - D. It turns a different color**
- 2. Why is the stability of GFP important in research?**
 - A. It affects the pH level**
 - B. It influences the energy consumption**
 - C. It determines the duration of observable fluorescence**
 - D. It impacts molecular interactions**
- 3. What occurs when lysate is applied to the column in a high salt buffer?**
 - A. Hydrophobic proteins pass through the column**
 - B. Hydrophobic proteins stick to the beads**
 - C. All proteins are washed out**
 - D. The column becomes saturated**
- 4. What role does arabinose play in the expression of the GFP gene?**
 - A. It acts as a preservative**
 - B. It inhibits protein production**
 - C. It induces the expression of the gene**
 - D. It alters the pH of the medium**
- 5. Which property of GFP can be altered through modifications for specific applications?**
 - A. Color**
 - B. Viscosity**
 - C. Molecular weight**
 - D. Solubility**

- 6. What should be used to transfer the bacterial culture into the centrifuge tube?**
- A. A spoon**
 - B. A syringe**
 - C. A clean pipette**
 - D. A dropper**
- 7. How does the Binding Buffer assist in the process of GFP purification?**
- A. It raises the temperature of the solution**
 - B. It decreases the salt concentration**
 - C. It enables hydrophobic proteins to bind**
 - D. It stabilizes the GFP structure**
- 8. What volume of TE buffer is added to the column in Step 10?**
- A. 250 uL**
 - B. 500 uL**
 - C. 750 uL**
 - D. 1000 uL**
- 9. In which step of the process are the bacteria resuspended?**
- A. Step 5**
 - B. Step 6**
 - C. Step 7**
 - D. Step 4**
- 10. What feature allows for specific interaction during nickel affinity chromatography with His-tagged GFP?**
- A. The charge of the protein**
 - B. The three-dimensional conformation**
 - C. The histidine residues present in the tag**
 - D. The molecular weight of the GFP**

Answers

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

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Explanations

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1. What occurs to GFP when incubated in a high salt Binding Buffer?

- A. It precipitates out of solution**
- B. It undergoes conformational change**
- C. It remains completely soluble**
- D. It turns a different color**

When GFP is incubated in a high salt Binding Buffer, it experiences a conformational change. High salt concentrations can affect the electrostatic interactions that maintain the protein's structure. Specifically, the ions in the salt solution can shield the charges on the protein, leading to a reduction in stabilizing interactions. This may result in changes to the folding or arrangement of the polypeptide chain, thus affecting the overall conformation of GFP. Conformational changes are significant because they can influence the protein's functionality, stability, and ability to interact with other molecules, which is critical during purification processes.

2. Why is the stability of GFP important in research?

- A. It affects the pH level**
- B. It influences the energy consumption**
- C. It determines the duration of observable fluorescence**
- D. It impacts molecular interactions**

The stability of GFP is crucial in research primarily because it determines the duration of observable fluorescence. When GFP is stable, it can resist denaturation and maintain its fluorescent properties over time and under various experimental conditions. This prolonged fluorescence is essential for accurately tracking cellular processes, visualizing protein expression, and conducting long-term imaging studies. If the fluorescent protein is not stable, the intensity and reliability of the fluorescence signal can diminish, leading to challenges in interpreting results and making it difficult to draw clear conclusions from experiments. Therefore, the stability of GFP directly correlates with its effectiveness as a research tool in various biological applications.

3. What occurs when lysate is applied to the column in a high salt buffer?

- A. Hydrophobic proteins pass through the column**
- B. Hydrophobic proteins stick to the beads**
- C. All proteins are washed out**
- D. The column becomes saturated**

When lysate is applied to the column in a high salt buffer, hydrophobic proteins tend to stick to the beads. This is due to the principle of hydrophobic interaction chromatography, where the beads in the column are often coated with hydrophobic materials. In a high salt environment, proteins are encouraged to expose their hydrophobic regions because the salt reduces the solubility of these hydrophobic portions in the aqueous buffer. This promotes interaction between the hydrophobic regions of the proteins and the hydrophobic beads, resulting in the binding of hydrophobic proteins to the column while more polar or hydrophilic proteins will typically remain in the mobile phase and wash through the column. In summary, the high salt buffer enhances the attraction between hydrophobic proteins and the column's beads, leading to effective trapping of these proteins and allowing for subsequent purification steps. This answer aligns with the principles of protein purification techniques, providing a clear rationale for why hydrophobic proteins are retained in the column under these conditions.

4. What role does arabinose play in the expression of the GFP gene?

- A. It acts as a preservative**
- B. It inhibits protein production**
- C. It induces the expression of the gene**
- D. It alters the pH of the medium**

Arabinose serves as an inducer for the expression of the Green Fluorescent Protein (GFP) gene in a system utilizing the pBAD promoter. When arabinose is added to the growth medium, it interacts with the regulatory proteins that are responsible for controlling the pBAD promoter. This interaction allows for the transcription of the GFP gene to initiate, leading to the production of GFP in the cells. The process is dependent on arabinose because, in the absence of arabinose, the promoter remains inactive, and GFP is not expressed. In this context, the other options do not accurately reflect the role of arabinose. It is not a preservative, nor does it inhibit or alter the pH of the medium in a way relevant to GFP expression. Instead, its primary function is to stimulate the transcription of the GFP gene, thereby allowing for the desired protein production.

5. Which property of GFP can be altered through modifications for specific applications?

- A. Color**
- B. Viscosity**
- C. Molecular weight**
- D. Solubility**

The correct choice highlights the fact that the color of Green Fluorescent Protein (GFP) can indeed be altered through various biochemical modifications. Different variants of GFP have been developed that emit fluorescence at different wavelengths, which allows for a spectrum of colors from blue to red depending on the specific modifications made to the protein's structure. These alterations are typically achieved through genetic engineering techniques, where changes in the amino acid sequence can create new chromophores or modify existing ones. This flexibility in color manipulation makes GFP extremely useful in molecular biology and biochemistry, as it enables researchers to utilize multiple fluorescent markers in a single experiment for parallel tracking of different proteins or cellular processes. By incorporating variations of GFP with distinct emission spectra, scientists gain the ability to label and visualize multiple targets within a biological system. While viscosity, molecular weight, and solubility are important properties that can affect the behavior of proteins, they are not as easily modified for application-specific purposes as the color of GFP. Adjusting these properties can be more challenging and often does not have the same direct application in fluorescent tagging.

6. What should be used to transfer the bacterial culture into the centrifuge tube?

- A. A spoon**
- B. A syringe**
- C. A clean pipette**
- D. A dropper**

The use of a clean pipette to transfer the bacterial culture into the centrifuge tube is appropriate because it allows for precise and controlled dispensing of liquids, which is crucial in laboratory settings to avoid contamination and ensure accurate measurements. Pipettes are designed for handling small volumes of liquids, ensuring that the transfer is efficient and minimizing the risk of spills or exposure to the environment. In microbiological techniques, maintaining sterility is extremely important to prevent contamination that could affect the results of experiments. Using a pipette maximizes this sterility compared to other options, as it can be single-use or properly sterilized before use. Spoons, syringes, and droppers may not provide the same level of precision or sterility required for transferring bacterial cultures, which can lead to inconsistent volumes or contamination of the sample. Thus, choosing a clean pipette is the best practice for such tasks.

7. How does the Binding Buffer assist in the process of GFP purification?

- A. It raises the temperature of the solution**
- B. It decreases the salt concentration**
- C. It enables hydrophobic proteins to bind**
- D. It stabilizes the GFP structure**

The Binding Buffer plays a crucial role in the purification of GFP by facilitating the binding of hydrophobic proteins, including GFP, to the affinity matrix used during purification. The buffer is designed to create conditions that promote the interaction between the hydrophobic regions of the protein and the hydrophobic environment of the binding medium, such as beads or chromatography resins that are used in the purification process. This hydrophobic interaction is essential because it allows the GFP to adhere to the matrix, effectively separating it from other components present in the solution. Once the GFP is bound, washing and elution steps can be conducted to purify the protein based specifically on those interactions, leading to a higher purity of the target protein. The buffer's composition and pH are optimized to ensure that GFP maintains its functional conformation while effectively engaging in these necessary interactions for purification.

8. What volume of TE buffer is added to the column in Step 10?

- A. 250 uL**
- B. 500 uL**
- C. 750 uL**
- D. 1000 uL**

The volume of TE buffer added to the column in Step 10 is 750 uL. This specific volume is important as it ensures an adequate buffer exchange, facilitating the elution of proteins from the column while maintaining their stability and functionality. The choice of volume is typically determined based on the column size and the amount of protein being purified, ensuring effective interaction with the buffer to achieve optimal purification results. Using the appropriate volume of TE buffer is key for proper rinsing and elution of the target protein, in this case, Green Fluorescent Protein (GFP), which helps in achieving a concentrated and pure sample for subsequent analysis or applications. The other volumes do not provide the same level of efficiency in this context, either being too small to effectively wash the column or too large, leading to unnecessary dilution of the protein sample.

9. In which step of the process are the bacteria resuspended?

- A. Step 5
- B. Step 6
- C. Step 7**
- D. Step 4

In the GFP purification process, the bacteria are resuspended in Step 7. This step typically occurs after cell lysis, which is crucial for releasing the GFP from the bacteria's cellular structure. Resuspension involves taking the cell pellet, which contains the lysed cells and their proteins, and mixing it in a suitable buffer to ensure that the GFP and other proteins are in a homogenous solution. This preparation is essential for the following purification techniques, such as centrifugation or chromatography, allowing the GFP to be isolated effectively based on its properties. Resuspending the bacteria correctly is fundamental for the overall success of the purification process.

10. What feature allows for specific interaction during nickel affinity chromatography with His-tagged GFP?

- A. The charge of the protein
- B. The three-dimensional conformation
- C. The histidine residues present in the tag**
- D. The molecular weight of the GFP

The specific interaction during nickel affinity chromatography with His-tagged GFP is primarily due to the histidine residues present in the tag. His-tags are short sequences of histidine amino acids that are incorporated into the protein. Nickel ions can bind to the imidazole side chains of histidine, creating a strong interaction. This property is utilized in affinity chromatography, where the His-tagged protein is effectively retained on a nickel-coated resin while other non-tagged proteins are washed away. This allows for a highly selective purification process, enabling the isolation of GFP based on the presence of the His-tag with its unique ability to coordinate with nickel ions. While other options mention factors like charge, conformation, and molecular weight, they do not specifically relate to the mechanism of interaction occurring in nickel affinity chromatography, which relies fundamentally on the presence of histidine residues in the tag for binding to the nickel ions. Therefore, the correct answer highlights the essential role of the histidine residues in this specific purification technique.