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

Study Guide

BY EXAMZIFY

Everything you need from our exam experts!

Questions

1. **Why is it important to carry out the purification in a sterile environment?**
   - A. It accelerates the purification process
   - B. It reduces costs associated with purification
   - C. It prevents contamination that can affect the results
   - D. It increases the yield of purified GFP

2. **What is the total volume of culture transferred to the microcentrifuge tube in Exercise 1?**
   - A. 1mL
   - B. 2mL
   - C. 0.5mL
   - D. 3mL

3. **During which step of GFP purification is a lysis buffer typically used?**
   - A. Elution phase
   - B. Cell disruption phase
   - C. Filtration phase
   - D. Concentration phase

4. **Which characteristic of GFP makes it particularly suitable for purification?**
   - A. It contains no hydrophobic amino acids.
   - B. It is less hydrophobic than most bacterial proteins.
   - C. It has many hydrophobic amino acids.
   - D. It is larger than other bacterial proteins.

5. **What must be done immediately after centrifugation of the microcentrifuge tube?**
   - A. Let the tube cool
   - B. Remove the tube from the centrifuge
   - C. Fill the tube with buffer
   - D. Shake the tube vigorously

6. Why is the pellet discarded during the purification procedure?

A. It is too heavy

B. It contains unwanted proteins

C. It does not contain GFP

D. It is too small

7. What type of buffer is the Equilibrium Buffer (EB) in the chromatography process?

A. A very low salt buffer

B. A medium salt buffer

C. A high salt buffer

D. A neutral pH buffer

8. 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

9. How do variations in the GFP structure impact its fluorescence?

A. They have no effect on fluorescence

B. They can affect chromophore formation, stability, and fluorescence properties

C. They only improve the stability of the protein

D. They exclusively decrease the brightness of fluorescence

10. What is an advantage of calibrating fluorescence detection equipment?

A. It ensures higher resolution

B. It allows for faster data collection

C. It guarantees consistent results across experiments

D. It simplifies the analysis process

Answers

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

Explanations

1. Why is it important to carry out the purification in a sterile environment?

A. It accelerates the purification process

B. It reduces costs associated with purification

C. It prevents contamination that can affect the results

D. It increases the yield of purified GFP

Carrying out the purification of Green Fluorescent Protein (GFP) in a sterile environment is crucial primarily because it prevents contamination that can affect the results. Sterility is essential in any biochemical process to ensure that no unwanted microbes, enzymes, or proteins interfere with the purification protocol. Contamination can lead to misleading data, compromised experiments, and ultimately produce unreliable protein samples. By maintaining a sterile environment, researchers can ensure that they are isolating only the intended GFP, thereby obtaining accurate and reproducible results. While other factors, such as cost and yield, may be influenced by the overall process, the primary concern during purification is to maintain the integrity of the sample. Ensuring sterility safeguards the quality of the GFP produced, making option C the most pertinent answer for understanding the importance of a sterile environment in the purification process.

2. 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.

3. During which step of GFP purification is a lysis buffer typically used?

A. Elution phase

B. Cell disruption phase

C. Filtration phase

D. Concentration phase

The lysis buffer is crucially used during the cell disruption phase of the GFP purification process. In this phase, cells containing the green fluorescent protein are subjected to conditions that break down their membranes, releasing their contents into the solution. The lysis buffer contains various components, such as detergents, salts, and sometimes enzymes, that facilitate the breakdown of the cell membrane and help to solubilize the target proteins. Using a lysis buffer is essential for maximizing protein yield, as it ensures that the GFP is extracted effectively from the cellular environment. The buffer also often includes protease inhibitors to prevent the degradation of proteins during this process. This step is foundational for the subsequent purification processes, as it directly influences the quality and quantity of the GFP available for further purification stages.

4. Which characteristic of GFP makes it particularly suitable for purification?

A. It contains no hydrophobic amino acids.

B. It is less hydrophobic than most bacterial proteins.

C. It has many hydrophobic amino acids.

D. It is larger than other bacterial proteins.

Green Fluorescent Protein (GFP) is particularly suitable for purification due to its abundance of hydrophobic amino acids. These hydrophobic regions promote strong interactions with certain types of chromatography media, such as hydrophobic interaction chromatography (HIC). During the purification process, these interactions can be effectively utilized to separate GFP from other proteins and contaminants in a mixture. The hydrophobic areas of GFP enable it to bind to the hydrophobic support in the column while less hydrophobic proteins will not bind as strongly or at all, allowing for a more efficient purification process. The hydrophobic properties of GFP not only facilitate its binding to specific matrices but also help in stabilizing its structure, which is crucial for maintaining its fluorescent properties throughout the purification process. This characteristic of having many hydrophobic amino acids directly influences its behavior during purification, making it easier to isolate from a complex mixture of other proteins.

5. What must be done immediately after centrifugation of the microcentrifuge tube?

A. Let the tube cool

B. Remove the tube from the centrifuge

C. Fill the tube with buffer

D. Shake the tube vigorously

After centrifugation of the microcentrifuge tube, it is essential to remove the tube from the centrifuge promptly. This action is crucial because leaving the tube in the centrifuge can increase the risk of sample contamination and degradation, especially if the sample contains sensitive biomolecules like proteins or nucleic acids. Additionally, if the tube is not removed in a timely manner, gravitational forces can cause the material in the tube to settle back down, potentially compromising the results of the experiment. Once the tube is removed, other steps can be taken, such as cooling, filling with buffer, or shaking, but the priority is to handle the tube immediately afterward to maintain sample integrity and ensure accurate results.

6. Why is the pellet discarded during the purification procedure?

A. It is too heavy

B. It contains unwanted proteins

C. It does not contain GFP

D. It is too small

The pellet is discarded during the purification procedure primarily because it does not contain GFP, which is the target protein. During the purification process, cells are typically lysed to release their contents, and subsequent centrifugation helps separate the soluble proteins from the insoluble cellular debris, which accumulates as a pellet. The soluble fraction, where GFP is located, is retained for further purification steps. The pellet often contains cell membranes, inactive proteins, and other unwanted cellular components that are not of interest for GFP purification. Since the goal is to isolate and purify the GFP, the pellet that lacks the target protein is discarded to ensure that only the fractions containing GFP are further processed. This efficient separation process is crucial in obtaining a high-purity yield of GFP for downstream applications or analysis.

7. What type of buffer is the Equilibrium Buffer (EB) in the chromatography process?

A. A very low salt buffer

B. A medium salt buffer

C. A high salt buffer

D. A neutral pH buffer

The Equilibrium Buffer (EB) used in the chromatography process is characterized as a high salt buffer. In protein purification, particularly when dealing with the chromatographic techniques such as affinity or ion exchange chromatography, the concentration of salt in the buffer plays a crucial role in maintaining protein solubility and stability. A high salt buffer helps to promote the proper folding of proteins by shielding charges on the protein's surface, which can prevent aggregation or non-specific interactions that might lead to erroneous results during the purification process. Additionally, in techniques such as ion exchange chromatography, the ionic strength of the buffer can significantly influence the binding and elution of the target protein; higher salt concentrations help to elute bound proteins by disrupting their interactions with the resins. This understanding of the role of salt concentration in buffers is critical for effectively optimizing protein purification protocols, which is why identifying the type of buffer correctly is vital for successful experimentation.

8. 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.

9. How do variations in the GFP structure impact its fluorescence?

A. They have no effect on fluorescence

B. They can affect chromophore formation, stability, and fluorescence properties

C. They only improve the stability of the protein

D. They exclusively decrease the brightness of fluorescence

Variations in the structure of Green Fluorescent Protein (GFP) can significantly impact its fluorescence properties. The fluorescence of GFP is primarily dependent on the chromophore, which is the part of the molecule responsible for its ability to absorb light and emit fluorescence. Alterations in the protein's structure can influence the formation and stability of this chromophore, which in turn can affect the intensity, color, and overall fluorescence characteristics. For instance, any mutations or modifications in the amino acid sequence surrounding the chromophore can lead to changes in how light interacts with it. This could result in altered fluorescence emissions, such as changes in brightness or shifts in wavelength. Enhancements in structural stability may also affect how efficiently the chromophore can fluoresce, as less stable forms may not be able to maintain the appropriate environment necessary for effective light absorption and emission. Overall, understanding these variations and their effects on fluorescence is crucial for applications in biotechnology and molecular biology, where GFP is often used as a marker or indicator in various experiments. This makes the influence of structural variations on the chromophore and fluorescence properties particularly important.

10. What is an advantage of calibrating fluorescence detection equipment?

A. It ensures higher resolution

B. It allows for faster data collection

C. It guarantees consistent results across experiments

D. It simplifies the analysis process

Calibrating fluorescence detection equipment is crucial because it ensures consistent results across experiments. Calibration involves adjusting the equipment to account for variations in light output, detector sensitivity, and other factors that may influence readings. By establishing a reliable baseline and correcting for these discrepancies, researchers can achieve reproducible and reliable data. Consistency in fluorescence measurements is essential in experiments, as it helps validate findings and allows for meaningful comparisons between different experimental conditions or studies. The other options, while they may seem relevant to some aspects of scientific experimentation, do not specifically address the fundamental purpose of calibration. Calibration primarily focuses on standardizing measurements to ensure reliability, rather than improving resolution, speed of data collection, or simplifying the analysis process. These aspects might be beneficial in their own rights, but they are not direct outcomes of the calibration process itself.