

Biochem Lab Exam 2 Review

Experiment 4- Purification of rGFP using Ni+2 Agarose

- 1) **Underlying principle that allows for the purification of rGFP (how to purify rGFP)?**
 - Exploiting the physical difference between your molecule of interest and all other molecules.
 - Chromatography separates proteins via differential interactions with both a mobile and stationary phase.
- 2) **Why does freezing and then thawing the bacterial pellet result in lysis?**
 - freezing and thawing to make crude extract
 - From freezing, ice crystals form, that break open neighboring cell walls.
 - Thawing helps make it more efficient because from damaged bacteria, the cytoplasm is released in the breaking buffer. This lysozyme will lyse neighboring bacteria and begin a chain reaction on lysing
- 3) **Purpose of the "wash" step when developing the column?**
 - the washes pull out any contaminants and basically any proteins that aren't His6-tagged (rGFP) that don't bind to the column.
- 4) **How are we monitoring the presence of rGFP?**
 - by using handheld UV light to see presence...not amount! That would be using a fluorescent microplate reader. So the microplate reader show proteing amount and protein activity.

If we were purifying a histidine tagged phosphatase, how would we monitor its presence during the purification procedure?

 - Monitor presence by reaction with PPP (colorimetric assay)
- 5) **What is the underlying principle that allows for the elution of rGFP from the Ni+2 agarose column?**
 - Imidazole, which is in the elution buffer, is very similar to hisitdine and competes for the binding sites in the column. So basically, the his6 tags get released out of the column when imidazole comes in.
- 6) **What are the five major types of column chromatography and the underlying scientific principles that make them useful for separating proteins?**
 1. **Gel-filtration chromatography**- separates based on molecular weight
 2. **Ion exchange chromatography**- based on charge
 - a. Anion-exchange: binds negative charge
 - b. Cation-exchange: binds positive charge
 3. **Hydrophobic interaction chromatography**- based on hydrophobic properties
 4. **Affinity chromatography**- based on specific molecular binding properties.

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Donna Gosnell, William Coleman

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Clinical Biochemistry Peter Rae, Mike Crane, Rebecca Pattenden, 2017-10-02 Clinical Biochemistry Lecture Notes presents the fundamental science behind common biochemical investigations used in clinical practice Taking a system based approach it explores the underlying physiological rationale for tests with each test explained within the context of disruption by disease It also explores the value and limitations of biochemical investigations while helping readers to quickly develop the knowledge and skills required to select the appropriate investigations for diagnosis and management and to correctly interpret test results Case studies throughout chapters place the information within a clinical context to further assist readers in the development of test selection and interpretation skills Key features include A comprehensive yet concise overview of the science behind common biochemical investigations Helps readers rapidly acquire a fully integrated practical understanding of biochemical diagnostics Full colour flowcharts and algorithms detailing the rationale for tests the biochemical processes involved and test procedures for quick comprehension and reference More clinical cases demonstrating application to practice Now in its tenth edition this classic introductory reference and revision text is indispensable to medical students and all those who want to quickly acquire a practical understanding of the scientific principles underpinning biochemical tests and a working knowledge of test selection test procedures and the interpretation of results within a clinical context

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