National Exams December 2012

04-Bio-B10, Analytical Biochemistry

3 hours duration

## NOTES:

1. If doubt exists as to the interpretation of any question, the candidate is urged to submit with the answer paper, a clear statement of any assumptions made.

2. This is a CLOSED BOOK EXAM.
   Any Casio or Sharp approved calculator is permitted.

3. FIVE (5) questions constitute a complete exam paper.
   The first five questions as they appear in the answer book will be marked.

4. Each question is of equal value.

5. Most questions require an answer in essay format. Clarity and organization of the answer are important.

| Q | Mark |
|---|---|
| 1 | /20 |
| 2 | /20 |
| 3 | /20 |
| 4 | /20 |
| 5 | /20 |
| Total | /100 |

**Question 1: (20 marks total) SDS-PAGE**

1) (8 marks total) Figure 1 shows the results of a purification process (using Protein A-based affinity chromatography) analyzed by silver staining an SDS-PAGE gel.
   a. (4 marks) What is SDS and why is it used in the analysis of proteins by gel electrophoresis?
   b. (4 marks) What are the advantages of using a silver staining technique versus a Coomassie Brilliant Blue R250 staining technique?

2) (8 marks total) In Figure 1, the same volume of liquid has been added in each well for each lane prior to running the gel through electrophoresis.
   a. (4 marks) If our protein of interest has a molecular weight of approximately 45kDa, what can be said of our protein of interest in each lane?
   b. (4 marks) If the volumes of each eluent fraction were equal, what can be said of E1 and E2? If a third volume of eluent was passed through the column and then subsequently analyzed, what would you expect to see?

3) (4 marks) Gels are most used to give a qualitative result; however, as an engineer, you often want to have a quantitative result. What is densitometry and how is it used to give a quantitative result?

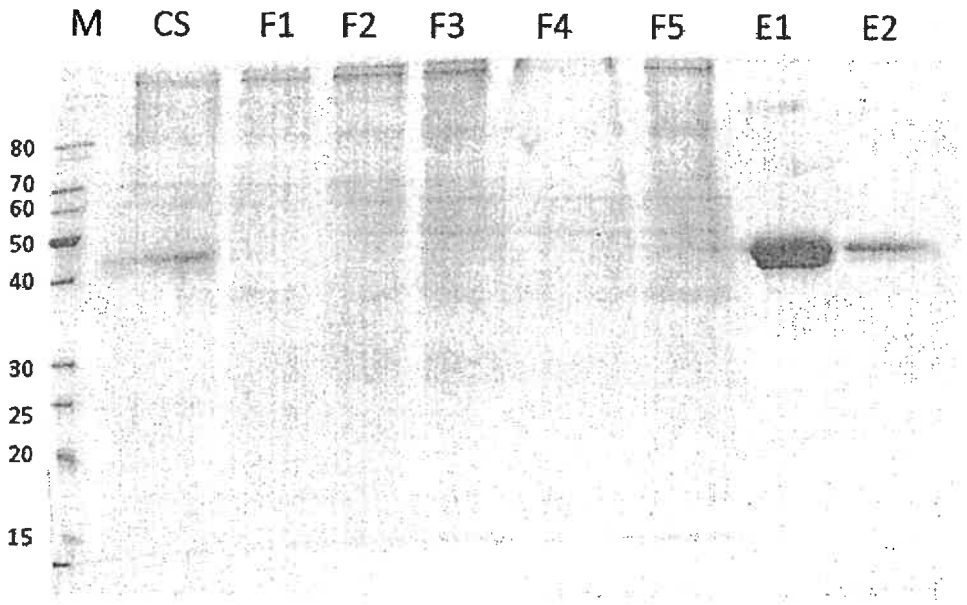


Figure 1: Silver stained SDS-PAGE gel of a protein purification process. M: molecular weight ladder (kDa). CS: culture supernatant. F1-F5: Fractions of flow-through material collected from loading step. E1-E2: Sequential fractions collected from the elution steps.

**Question 2: (20 marks total) Affinity Purification and Western Blot Analysis**

1) (5 marks) What type of protein can be captured using a Protein A-based affinity chromatography column? Why?

2) (5 marks) What steps form the basis of affinity-based purification?

3) (5 marks) A strategy for the production and purification of recombinant proteins is to allow the cell to add six histidine residues (polyhistidine-tag) at the end of the protein during the synthesis of the protein, making the protein have an increased affinity for _____ or _____.

4) (5 marks) In your own words, describe the similarities/dissimilarities of affinity purification and Western Blot analysis.

**Question 3: (20 marks total) HPLC**

1) (8 marks total) Ion exchange chromatography is widely used to separate biomolecules based on their charge. When doing analysis of biomolecules eluted from an anion-exchange column, one of your colleagues suggests you monitor the sample using UV light at 260 and 280 nm.
   a. (2 marks) What will bind to an anion-exchange column (i.e. what charge will the binding species carry?)
   b. (4 marks) What will these wavelengths give as information?
   c. (2 marks) What should the absorbance of the mobile phase be?

2) (4 marks) Another tool that can be used for examining the material coming out of the column is based on refractive index. What is the benefit of using a refractive index detector instead of a UV detector?

3) (4 marks) Size-exclusion chromatography is significantly different from ion-exchange or affinity chromatography. What principle is behind the separation of molecules using size-exclusion chromatography?

4) (4 marks) Explain in your own words what makes up a high performance liquid chromatography system. You may use a sketch to answer this question (make sure you label all diagrams).

**Question 4: (20 marks total) Polymerase Chain Reaction (PCR)**

1) (4 marks) What is the major non-biological component of a system used to perform polymerase chain reactions i.e. what is the major component of a thermal cycler? What are the major features of this component?

2) (4 marks) What are the major steps in a polymerase chain reaction? Describe what each step entails.

3) (2 marks) What are primers in polymerase chain reactions?

4) (8 marks total) In your own words, describe how you would:
   a. (4 marks) design an approach to use PCR to detect the levels (concentration) of a specific type of virus in a sample, i.e. what information and preparation would you need to set up the PCR reaction;
   b. (4 marks) you quantify and qualify your product post-reaction (note: there are many different answers to this question).

5) (2 marks) Figure 2 is a representative plot of what you would get from a real-time PCR analysis. The red curve can be viewed as a fluorescence signal increase from a single sample over a number of cycles. How would you use such a plot to estimate concentration of a product? Why is real-time PCR considered, by some, to be a better method for quantification over non-real-time PCR.

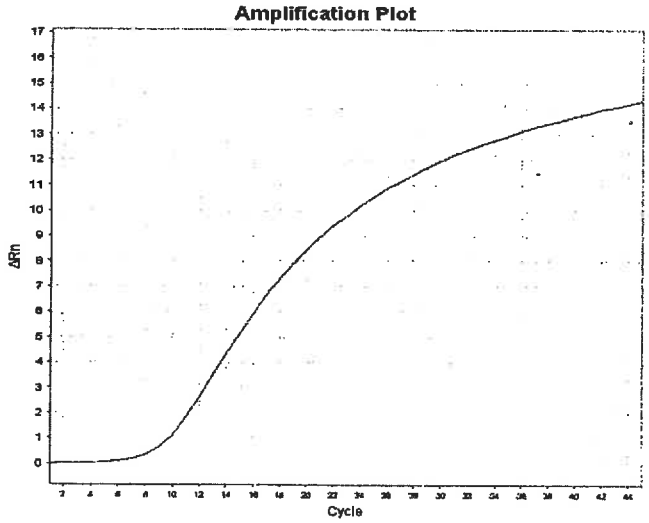


Figure 2: Amplification plot from a real-time PCR analysis of a gene from a virus.

**Question 5: (20 marks total) Flow Cytometry**

A B

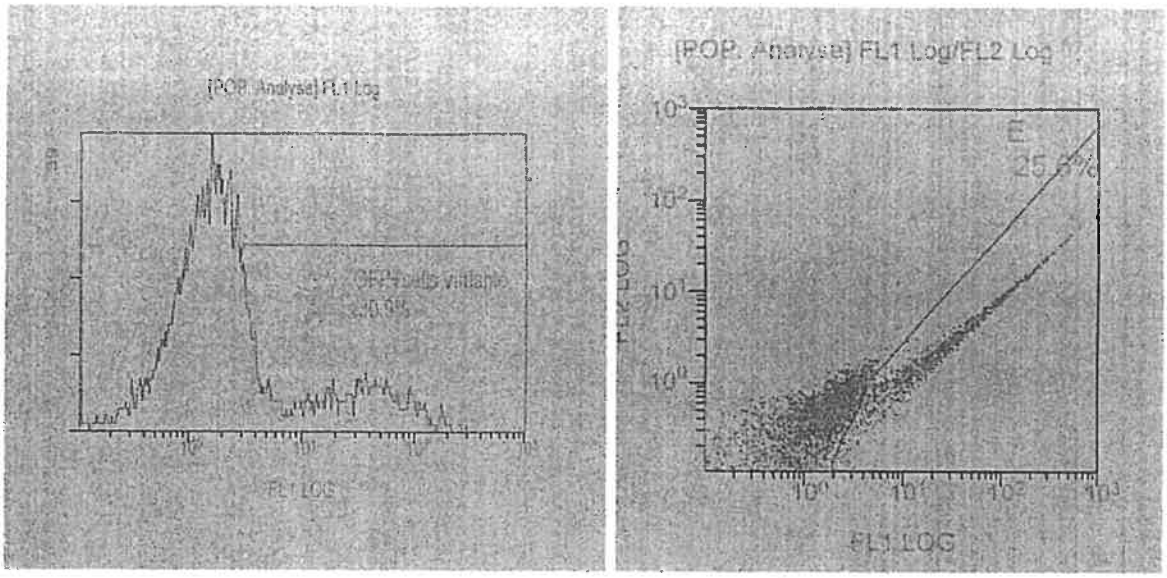


Figure 3: Typical results obtained from commercially available flow cytometers. A) Histogram of events (cells) emitting different fluorescent intensities (FL1LOG). Two populations of cells having different fluorescent properties can be detected. B) Scatter plot of events having different fluorescent emissions based on different laser excitations (FL2LOG vs FL1LOG). Similar to Figure A, two populations of cells having different fluorescent properties can be detected.

1) (3 marks) How many cells do you want to pass in front of the detector at once when analyzing cells using flow cytometry?

2) (3 marks) In flow cytometry, what is meant by hydrodynamic focusing?

3) (6 marks) In flow cytometric experiments, what is meant by forward and side scatter? What does each of these scattering results (forward and side) say about a cell?

4) (8marks) Explain in your own words, the relationship between flow cytometry and fluorescence activated cell sorting (FACS)

**Question 6: (20 Marks Total) Nuclear Magnetic Resonance (NMR)**

1) (6 marks) Name two atoms that are used in studying biological compounds by NMR spectroscopy? Why are these atoms important in biological studies? Why are they used in NMR?

2) (3marks) In your own words, describe what is meant by the resonance frequency?

3) (3 marks) What can be done to increase the resolution of NMR?

4) (5 marks) Describe two different applications of NMR in biological studies.

5) (3 marks) What is the minimum number of peaks you would expect to see from a 1 dimensional proton NMR experiment used to detect ethanol? Explain.

**Marking Scheme:**

**Question 1: (20 marks total) SDS-PAGE**

1) (8marks total)
    a. (4 marks)
    b. (4 marks)
2) (8 marks total)
    a. (4 marks)
    b. (4 marks)
3) (4 marks)

**Question 2: (20 marks) Affinity Purification and Western Blot Analysis**

1) (5 marks)
2) (5 marks)
3) (5 marks)
4) (5 marks)

**Question 3: (20 marks total) HPLC**

1) (8 marks total)
    a. (2 marks)
    b. (4 marks)
    c. (2 marks)
2) (4 marks)
3) (4 marks)
4) (4 marks)

**Question 4: (20 marks total) Polymerase Chain Reaction (PCR)**

1) (4 marks)
2) (4 marks)
3) (2 marks)
4) (8 marks total)
    a. (4 marks)
    b. (4 marks)
5) (2 marks)

**Question 5: (20 marks total) Flow Cytometry**

1) (3 marks)
2) (3 marks)
3) (6 marks)
4) (8 marks)

**Question 6: (20 Marks Total) Nuclear Magnetic Resonance (NMR)**

1) (6 marks)
2) (3 marks)
3) (3 marks)
4) (5 marks)
5) (3 marks)

Break-down of the marking scheme