

## National Exams December 2017

### 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.  
One of two calculators is permitted - any Casio or Sharp approved model.
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.*

**Question 1: (20 marks total) Polyacrylamide Gel Electrophoresis (PAGE)**

An immunoglobulin G (IgG) antibody consists of four subunits: two heavy chain and two light chain peptides.

a) (4 marks) What is the premise behind PAGE analysis?

b) (4 marks) When analyzing a purified preparation of the IgG using PAGE, should you use a silver staining technique or a Coomassie Brilliant Blue R250 staining technique? Why?

c) (4 marks) Why is sodium dodecyl sulfate (SDS) often integral to PAGE analysis?

d) (4 marks) After running the electrophoresis and visualizing your gel, you notice two (and only two) dark bands appear in the same lane. What preparation steps did you do to achieve this result? What does this tell you about the initial purified protein?

e) (4 marks) Suggest another protocol (set of steps) that could lead to a different banding pattern on your gel?

**Question 2: (20 marks total) Polymerase Chain Reaction (PCR)**

- a) (5 marks) Explain two features of a DNA template that could lead to complications in its amplification by PCR.
- b) (10 marks) A PCR is run for 20 cycles to amplify two different strands of DNA in the same sample. At the end of the 20 cycles, the DNA template with the lower initial copy number ended up with the greater amount of DNA copies. Is this possible? Explain.

- c) (5 marks) If the initial copy number of your template DNA was 7, how many strands of DNA would you expect to have after 10 PCR cycles? Explain any and all assumptions.

**Question 3: (20 marks total) Flow cytometry**

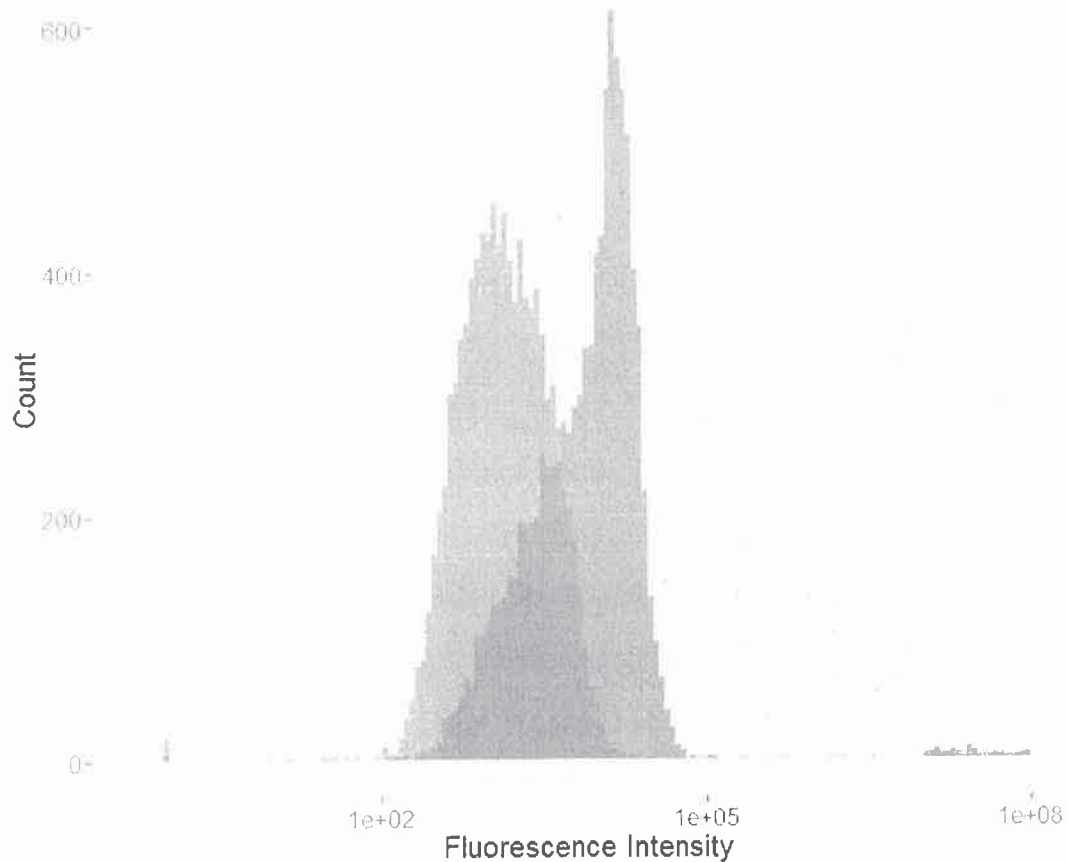


Figure 1: Histogram of fluorescent intensity for two samples (histograms are overlaid one on top of the other). Each sample contained 10000 events (whole cells). The sample represented in pink contained unlabeled cells. The sample represented in blue contained cells that were mixed with a fluorescently labeled antibody which targets a cell surface receptor.

In order to study the effect of a cell surface receptor, the presence of the receptor is probed using a fluorescently labeled antibody specific for the receptor in question.

a) (10 marks) Based on Figure 1, what can be said about the presence or absence of the receptor on the cells being investigated? Explain.



b) (5 marks) What properties of a cell are typically observed by flow cytometry?

c) (5 marks) When performing a flow cytometric experiment, such as the one presented in this question, what “control samples” should be used? Explain.

**Question 4: (20 Marks Total) High performance liquid chromatography (HPLC)**

- a) (5 marks) Ion exchange chromatography is one of the most utilized methods in high performance liquid chromatography. What is meant by a “strong” ion exchange matrix?
- b) (5 marks) Sketch out what occurs during the loading and elution steps when using a cation exchange membrane. Label the diagram appropriately.
-

c) (5 marks) What is gel permeation chromatography?

d) (5 marks) What is achieved when using two orthogonal chromatography methods to resolve a particular type of protein?

**Question 5: (20 marks total) Nuclear Magnetic Resonance (NMR) Spectroscopy**

- a) (6 marks) What are the major steps in an NMR spectroscopy experiment? Explain.

- b) (6 marks) Why is hydrogen a target for NMR spectroscopy?  
Explain.

c) (4 marks) What is achieved by using increasing magnetic fields in NMR spectroscopy? Explain.

d) (4 marks) What is the minimum magnetic field that can be used for NMR spectroscopy? Explain.

**Question 6: (20 marks total) Fourier Transform Infrared (FTIR)  
Spectroscopy**

a) (6 marks total)

i) (3 marks) What is a Fourier Transform?

ii) (3 marks) What is the role of the Fourier Transform in FTIR?



b) (6 marks) What types of sample (including state of matter) can be analyzed by FTIR? Explain.

c) (8 marks) Provide an example of the use of FTIR spectroscopy and the type of information that can be recuperated from the analysis.