Second Semester Examination
Academic Session 2020/2021

July 2021

KAE445 – Bioanalysis

Duration: 2 hours

Please check that this examination paper consists of **NINE (9)** pages of printed material before you begin the examination.

Instructions:

Answer **FOUR (4)** questions only.

SECTION A: **COMPULSORY** Question.

SECTION B: Answer **THREE (3)** questions only.

If a candidate answers more than four questions, only the first four questions in the answer sheet will be graded.

Answer each question on a new page.

You may answer the questions either in Bahasa Malaysia or in English.

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SECTION A: COMPULSORY QUESTION

1. (a) Explain why the conventional analytical methods below are not suitable for analysis of biomolecules.

(i) Gas chromatography (GC)
(ii) Nuclear magnetic resonance (NMR)
(iii) Infrared spectroscopy (IR)

(6 marks)

(b) With the support of a diagram, describe how an indirect sandwich assay is performed using enzyme-linked immunosorbent assay (ELISA). Assuming that the sample to be analysed is an antibody.

(7 marks)

(c) In bioanalytical chemistry, soft ionisation methods of electrospray ionisation (ESI) and matrix-assisted laser desorption/ionisation (MALDI) are preferred compared to hard ionization methods. Explain the statement.

(3 marks)

(d) Briefly discuss **TWO** physicochemical properties of protein that can be employed for its separation and purification using high performance liquid chromatography (HPLC).

(4 marks)

(e) Describe the general procedures of conducting gel electrophoresis.

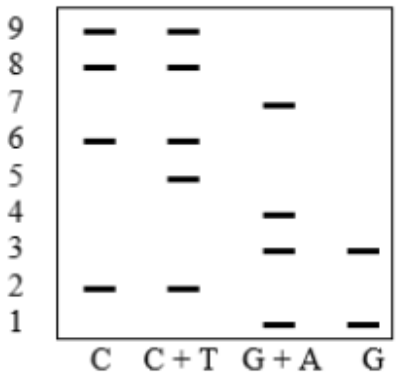
(5 marks)

SECTION B: ANSWER THREE (3) QUESTIONS ONLY

2. (a) Describe the working principles of Maxam Gilbert method in DNA sequencing.

(6 marks)

(b) Determine the DNA sequence of an unknown DNA sample below analysed by Maxam Gilbert method.



(3 marks)

(c) A pentapeptide was found to have the composition Ala, Arg, Gly, Pro, Trp. Reaction of the pentapeptide with Sanger's reagent, followed by hydrolysis, gave the DNP derivative of proline. Treatment of the pentapeptide with carboxypeptidase initially produced alanine. Treatment of the pentapeptide with trypsin gave a tetrapeptide which, when treated with chymotrypsin, produced a tripeptide.

(i) Determine the sequence of the pentapeptide.
(ii) Explain your answer.

(4 marks)

(d) An oligopeptide has the composition of Asn, 2Cys, Gln, Gly, Ile, Leu, Pro, Tyr. Partial hydrolysis of the oligopeptide led to the following fragments:
Asn-Cys
Cys-Tyr
Tyr-Ile-Gln
Cys-Pro-Leu
Ile-Gln
Leu-Gly
Gln-Asn-Cys

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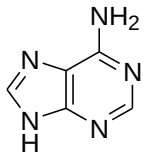
Reaction of the oligopeptide with carboxypeptidase showed glycine as the first liberated amino acid, while Sanger analysis gave the 2,4-dinitrofluorobenzene derivative of cysteine.

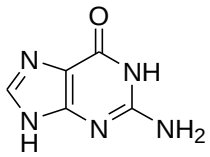
(i) Determine the sequence of the oligopeptide.
(ii) Show how you obtain your answer.

(4 marks)

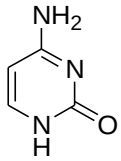
(e) Below are four nucleobases found in DNA. Draw the way they are connected to each other by hydrogen bonds to form base pairs.



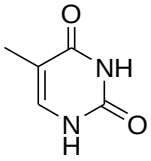
Adenine



Guanine



Cytosine



Tymine

(4 marks)

(f) Signal generation in immunoassays requires attachment of a label to one of the reagents. Fill in the blanks with correct answers.

Label	Example	Detection Method
Radioisotopes	(i)	Isotopic counting
Enzyme assays	(ii)	(iii)
(iv)	Luminol	Photomultiplier tubes

(4 marks)

3. (a) Outline the working principles of the real-time polymerase chain reaction (real-time PCR) using a probe-based Fluorescence Resonance Energy Transfer (FRET) assay.

(6 marks)

(b) The following reagents are often used in protein analysis. Match the reagent with the purpose for which it is best suited. Some answers may be used more than once or not at all; more than one reagent may be suitable for a given purpose.

Cyanogen bromide	Trypsin
Phenylisothiocyanate	Dithiothreitol
1-Fluoro-2,4-dinitrobenzene	Performic acid

(i) Hydrolysis of peptide bonds on the carboxyl side of Lys and Arg.

(ii) Cleavage of peptide bonds on the carboxyl side of Met.

(iii) Breakage of disulfide (—S—S—) bonds.

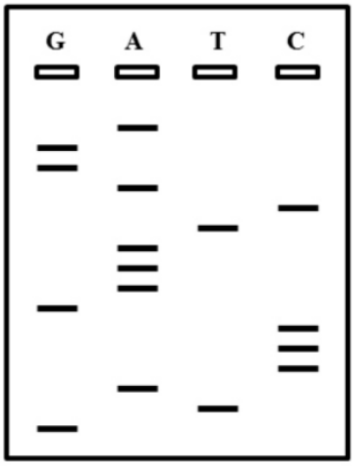
(iv) Determination of the amino acid sequence of a peptide.

(v) Determining the N-terminal amino acid in a polypeptide.

(6 marks)

(c) (i) Explain why 2',3'-dideoxynucleotides (ddNTPs) are used in the Sanger method of DNA sequencing.

(ii) Figure below shows the autoradiogram of the sequencing gel analysed using Sanger method. Write the sequence of the original DNA sample.



(6 marks)

(d) A peptide containing ten amino acids was analysed and found to contain the following amino acids (listed alphabetically) after complete hydrolysis.

Ala Arg Gly Ile Leu Lys Met Phe Ser Val

Treatment of the intact peptide with 2,4-dinitrofluorobenzene, followed by acid hydrolysis, yielded nine amino acids and leucine.

Treatment with the indicated reagents below, yielded peptides with the indicated compositions:

Cyanogen bromide: CB-1: Ala, Ile, Val
CB-2: Arg, Gly, Leu, Lys, Met, Phe, Ser

Chymotrypsin: CT-1: Leu, Lys, Phe
CT-2: Ala, Arg, Gly, Ile, Met, Ser, Val

Trypsin: T1: Leu, Lys
T2: Arg, Phe, Ser
T3: Ala, Gly, Ile, Met, Val

N-terminal analysis of peptide CB-1 revealed isoleucine.

Carboxypeptidase treatment of the peptide liberated a nine amino acid peptide and alanine.

(i) Determine the sequence of this peptide.
(ii) Show how you obtain your answer.

(7 marks)

4. (a) Table below shows physical characteristic of bovine whey proteins. The proteins were separated using reversed phase high performance liquid chromatography (RP-HPLC) using C18 column and mobile phase consisted of 95:5 % (v/v) (water: acetonitrile with 0.1% trifluoroacetic acid).

Protein	Molecular mass (kDa)	Amino acid residues
ß-lactoglobulin	19.88	178
α-lactalbumin	16.25	142
Serum albumin	69.29	607
Immunoglobulins	>150	>1300
Lactoferrin	78.06	708
Lactoperoxidase	80.64	712

(i) Describe the elution order of the proteins.

(ii) Predict the elution order of the proteins if hydrophobic interaction chromatography (HIC) is used for the separation of the proteins.

(iii) Explain your answer in (ii).

(iv) Discuss the specific approach to separate immunoglobulins from other proteins.

(15 marks)

(b) Protein binders in samples of a mid-18th century colonial mural painting in Bolivia was investigated using matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF/MS) and liquid chromatography electrospray tandem mass spectrometry (LC-ESI/MS/MS).

(i) Discuss why LC-ESI/MS/MS is more confirmative than MALDI-TOF/MS in the determination of protein binders in the mural painting.

(ii) Describe another technique could be used to determine the molecular weight of the protein binders.

(10 marks)

5. (a) The separation and identification of egg white proteins in the table below can be performed using different modes of electrophoresis.

Protein	Molecular mass (kDa)	Isoelectric point (pl)
Cystatin	12.7	5.1
Lysozyme	14.3	10.7
Ovalbumin	45	4.5
Ovoglobulin	49	5.5
Ovoinhibitor	49	5.1
Ovotransferrin	77.7	6.0

(i) Sketch the bands of the proteins if separated using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) on a 4-12% Bis-Tris gel and visualised using Coomassie brilliant blue.

(ii) Sketch the bands of the proteins if separated using isoelectric focusing (IEF) on IPG pH 3-10 strip.

(iii) Comment the efficiency of the separation of the proteins in (i) and (ii).

(iv) Describe another electrophoresis mode that can separate all the proteins.

(19 marks)

(b) Explain the effect of the following on the separation of proteins:

(i) agarose gel with small pore size is used in IEF,

(ii) when electrophoresis buffer used without sodium dodecyl sulphate (SDS), ß-mercaptoethanol and heat in SDS-PAGE.

(6 marks)

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