Student Code: _____

22nd INTERNATIONAL BIOLOGY OLYMPIAD

July 10-17, 2011

Taipei, Taiwan

PRACTICAL TEST 1

BIOCHEMISTRY AND CELL BIOLOGY

生物化學與細胞學

Total Points: 100

總分 100

Duration: 90 minutes

考試時間 90 分鐘

Dear Participants, 敬愛的參賽者

- In this test, you have been given the following 3 tasks: 本試卷包含三個實做題目

  Task I: Protein electrophoresis (35 points) 蛋白質電泳 (35 分)

  Task II: Protein quantification (30 points) 蛋白質定量 (30 分)

  Task III: Protein purification (35 points) 蛋白質純化 (35 分)

- Check your **Student Code** on the **Answer Sheet** before starting the test.

  檢查答案卷的號碼是否與你的考生編號相同。

- Write down your results and answers in the **Answer Sheet**. **Answers written in the Question Paper will not be evaluated.**

  一定要將答案填入答案卷上的正確位置，答案寫在試卷上將不予計分。

- Make sure that you have received all the materials listed for each task. If any of the listed items is missing, **raise your sign.**

  確認每題的材料與問題是相符的，如果有缺少任何物件，**請舉牌**。

- Use pen only. **不可使用鉛筆**

- You should organize your work efficiently but ensure that you complete task II early enough to obtain the spectrophotometer readings to answer the questions that follow.

  你可以根據實際狀況有效率的回答問題，以便完成 task II 中分光光度計的實驗，並回答之後的題目。

- Stop answering **immediately** after the end bell rings. 鈴響後**立刻**停止作答
- After test, enclose both the **Answer sheets, Question paper, and Data printout** in the provided envelope. Our lab assistants will collect it promptly.

  考試結束後，將**答案卷，試題卷與列印出的數據**放入指定的信封內。助教會迅速的收走。

- No paper or materials should be taken out of the laboratory.

  任何紙張與材料嚴禁攜出實驗室。

Good Luck!!

Shared instruments:共用設備

Camera, spectrophotometer, printer　照相機，分光光度計，印表機

Equipments and Materials:設備與材料

	Equipments: 設備	**Quantity**
1	Power supply　電源供應器	1
2	Electrophoresis tank (with gel and buffer) 電泳槽 (含膠體與緩衝液)	1
3	Micropipettes P20 and P200 微量吸管 P20, P200	1 each
4	80-well microcentrifuge tube rack　80 孔 微量離心管管架	1
5	Wire test tube rack with 15-mL centrifuge tubes (×6) (yellow cap) 不鏽鋼試管架包括 6 支黃蓋 15 mL 離心管	1
6	4-way test tube rack 四面試管架	1
7	Plastic droppers in 15-mL centrifuge tubes 15 mL 離心管附塑膠滴管	2
8	Micropipette tips (for P20 and P200)　微量吸管頭	1 each
9	Timer　計時器	1
10	96-well microplate　96 孔微量盤	1
11	Marker pen & paper label　奇異筆與貼紙	1 each
12	600-mL beaker for waste disposal　600 mL 燒杯 (裝廢液)	1
13	Scissors　剪刀	1
14	Double-sticker to attach the results　雙面膠 (貼結果用)	1
15	Student Code sticker　學生編號貼紙	1
16	Tissue paper　衛生紙	1
17	Mini centrifuge (if you need to spin down the samples in the microcentrifuge tubes)　微量離心機 (沉澱樣本用)	1

	Materials: 材料	**Quantity**
1	Loading dye (microcentrifuge tube-L) (pink tube with orange label) 指示染料 (微量試管-L)(粉紅色試管橙色標籤)	1
2	Pre-stained protein molecular weight marker (microcentrifuge tube-M) (pink tube with orange label) 預染蛋白質分子量指示標記 (微量試管-M)(粉紅色試管橙色標籤)	1
3	Unknown pre-stained protein samples (microcentrifuge tubes-U1 and U2) (pink tube with orange label) 未知預染蛋白質樣本 (微量試管-U1 與 U2)(粉紅色試管橙色標籤)	1
4	CBG reagent in 50-mL centrifuge tube CBG 試劑裝在 50mL 離心管中	1
5	Bovine serum albumin (BSA) concentration standard (0.5 mg/mL) in microcentrifuge tube (green tube with yellow label) 牛血清白蛋白 (BSA) 濃度測定標準樣本 (0.5 mg/ml) (綠色試管黃色標籤)	1
6	Enzyme E in two microcentrifuge tubes: concentrations X and Y (green tube with yellow label) 濃度 X 與 Y 的酵素 E (綠色試管黃色標籤)	1
7	Distilled water (microcentrifuge tube-ddH_2O) (green tube with yellow label) 蒸餾水 (微量試管 dd H_2O) (綠色試管黃色標籤)	1
8	Protein sample (microcentrifuge tube-C) (blue tube with blue label) 蛋白質樣本 (微量試管 C)(藍色試管藍色標籤)	1
9	Anion exchange chromatography column on 15-mL centrifuge tube 15mL 離心管附陽離子交換色層分析管	1
10	Anionic buffers A and B (5 mL each in two separated 15-mL centrifuge tubes) (green cap) 陽離子緩衝溶液 A 與 B (各 5 mL 分別裝於 15 mL 綠色蓋子離心管)	1
11	Coomassie brilliant blue G-250 (CBG) reagent 1 mL in each of six 15-mL centrifuge tubes (A1 to A3 & B1 to B3, red cap) Coomassie brilliant blue G-250 (CBG) 試劑，分別裝於 15 mL 紅色蓋子離心管，每管 1mL 並分別標記爲 A1, A2, A3, B1, B2, B3)	1

Task I (35 points)

Protein electrophoresis 蛋白質電泳

Introduction: 簡介

Polyacrylamide gel electrophoresis (PAGE) is a common technique for protein study. It can be used to separate different proteins based on their charges or sizes. A type of PAGE is termed SDS-PAGE, in which the negatively charged chemical, SDS, is added before protein electrophoresis. The amount of SDS that binds to proteins is proportional to the size of the protein which confers each protein a similar charge-to-mass ratio and renders the intrinsic charge of the protein insignificant, at least for this experiment. Thus, the major factor that affects the migration of protein is the molecular weight (MW) of the protein during SDS-PAGE. The relative mobility (R_f) of the protein can be calculated as the ratio of the distance migrated by the protein to that migrated by the dye-front. The value of R_f is inversely proportional to the log of its molecular weight.

聚丙醯胺膠體電泳 (Polyacrylamide gel electrophoresis; PAGE) 經常用於蛋白質研究。可以藉由蛋白質的電荷與分子量大小的差異進行蛋白質分離。一種稱爲 SDS-PAGE 的技術，便是在進行電泳前在蛋白質樣本先添加一種帶負電的化學藥品，SDS。蛋白質所能結合 SDS 的量與蛋白質的大小成比例關係。因此，所有的蛋白質均具有相類似的荷質比 (charge-to-mass ratio)，因此可以忽略蛋白質本身所帶的電荷。所以，在進行 SDS-PAGE 的實驗時，影響蛋白質移動速率的最大因素便剩下蛋白質本身的分子量 (MW)。蛋白質相對移動速率 (R_f) 便可以藉由蛋白質移動距離與染料指示劑前緣的移動距離比值求出，而且 R_f 值與蛋白質分子量的對數值成比例關係。

In the problem set, you will perform the following experiment:

依序操作下列實驗並回答問題

1. An electrophoresis tank has been set up for SDS-PAGE, in which a polyacrylamide gel has been secured on electrode assembly and electrophoresis buffer has been filled. There are 10 wells for sample loading on the top of the gel. To load the sample, use the P20 micropipette with tip to withdraw protein sample, and carefully place the tip on the top of the well. By injecting slowly the sample will sink to the bottom of the well by gravity (**Figure 1**).

   一個已經確保可以進行實驗的 SDS-PAGE 電泳槽已經裝置完畢，同時已經添加完成電泳緩衝液。膠體中具有 10 個 well 供實驗所用。參考**圖 1** 所示位置，以 P20 的微量吸管吸取蛋白質樣本，置於 well 上端，並利用重力緩緩將樣本加入 well 中。

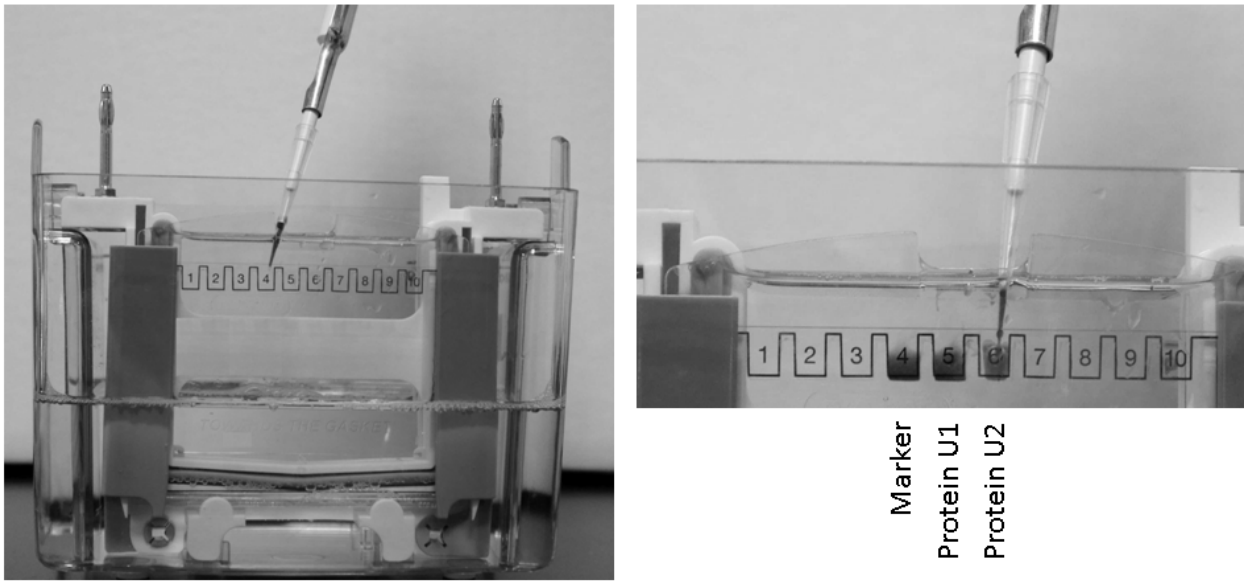

圖 1

2. If you need to practice, use the P20 micropipette with tip to withdraw 10 μL of loading dye from microcentrifuge tube L (pink tube with orange label) on rack. Load the dye into wells 1 to 3 or 7 to 10.

   如果需要練習，可以以 P20 微量吸管吸取 10 μL 微量離心管 L (粉紅色試管橙色標籤)的指示染料於編號 1 – 3 或 7 – 10 的 well。

3. Each of the microcentrifuge tubes M, U1 and U2 (pink tube with orange label) contains 15 μL of protein molecular weight marker, unknown protein U1 and unknown protein U2, respectively. Use micropipette P20 to withdraw 10 μL solution from each tube and load the samples into wells 4 to 6 as shown in **Figure 1**.

   利用 P20 微量吸管分別自含有 15μL 溶液的微量離心管中 (粉紅色試管橙色標籤) 吸取編號 M，U1，U2 的樣本各 10 μL，分別添加到 4 – 6 號 well 中，如**圖 1** 所示。

4. As soon as you finish sample loading, **Lift the sign**, lab assistants will connect the power cord to power supply and set the voltage to 200 V for you. The gel will run for 25 minutes. The timer will be set up by an assistant to countdown.

   完成上述實驗步驟 3 時，**請舉牌**。助教會協助你將電源供應器的電壓調到 200 V，並連接電泳槽到電源供應器的連接線。電泳時間爲 25 分鐘，助教會協助你操作計時器以利時間倒數。

5. After finishing electrophoresis, **Lift the sign**, lab assistants will disassemble the electrophoresis set-up and give back your gel. Wipe clean the surface of gel with tissue papers and **label the gel with your Student Code** sticker. Lab assistants will take the photo of your gel. Put the photo on the answer sheet using double-sticker (5 points).

   電泳完成後，**請舉牌**。助教會協助你卸下電泳槽，並取出膠體。用衛生紙擦拭膠體表面後，**貼上你的考生編號貼紙**，助教會拍下你的電泳膠體。將結果照片利用雙面膠帶貼在答案紙上 (5 分)。

Answer the following questions: 回答下列問題

Q.1.1. (2 points)

Figure 2 shows a photograph of a SDS-PAGE gel. The electrophoresis start point and dye-front are indicated. Which side of the gel should be connected to the anode (+ charge) of the power supply? Mark your answer (X) on the answer sheet.

圖 2 爲 SDS-PAGE 的結果。起始點與指示劑前緣如標記所示。請問哪一端要接到電源供應器的陽極 (＋極)？請在正確的答案處以 X 標記。

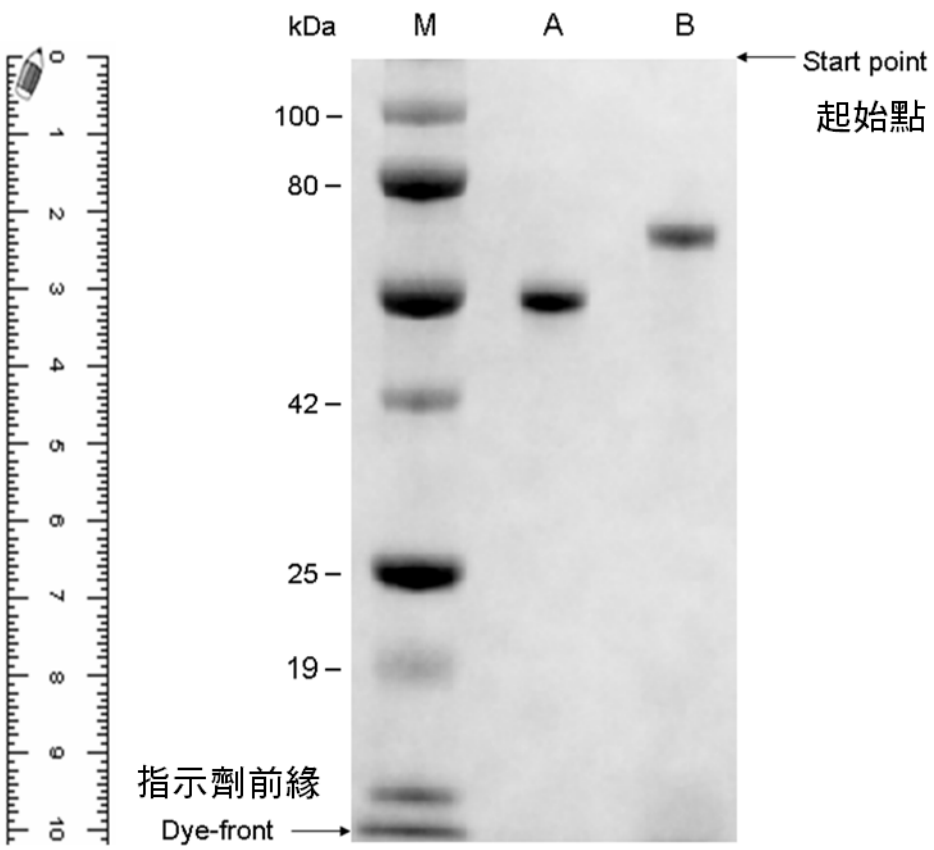

圖 2

Q.1.2. (8 points)

Based on the information provided in **Figure 2**, make a plot of log molecular weight values of the five marker proteins versus their relative migration-R_f values on the answer sheet (4 points). Use the graph to estimate the molecular weights of unknown proteins on lanes A and B (4 points). Write down your answers on the answer sheet.

根據**圖 2** 所提供的資訊，將選取 5 個蛋白質分子量指示標記分子量的對數值與相對移動速率值 (R_f) ，在答案紙上所提供的方格紙上作圖。(4 分)

利用你所畫出來的圖形，對未知蛋白質 A 與 B 進行分子量的預測。(4 分) 並將正確答案寫在答案紙上。

Q.1.3. (5 points)

A protein complex of molecular weight 246 kDa is composed of multiple subunits bound by non-covalent interaction. Two protein bands of 57 and 33 kDa were identified after SDS-PAGE. How many 57-kDa and 33-kDa subunits, respectively, are included in the protein complex? Write down your answers on the answer sheet.

一個分子量爲 246 kDa 的蛋白質複合物，該蛋白質由許多的次單元所構成，但都非共價鍵結合。經過 SDS-PAGE 分離後出現兩條分子量分別爲 33 kDa 與 57 kDa 的蛋白質帶。該蛋白質複合物分別由多少個 33 kDa 與 57 kDa 的蛋白質單元所構成？請將正確答案寫在答案紙上。

Q.1.4. (5 points)

The average molecular weight of amino acid residues is about 110 daltons. How many amino acids are there in the 33-kDa protein subunit? How many nucleotides of RNA are translated into the protein? Write down your answers on the answer sheet.

假設胺基酸的平均分子量爲 110 Da，請問一個 33 kDa 的蛋白質分子由多少個胺基酸所構成？參與此蛋白質分子轉譯的 RNA 含有多少個核苷酸？請將正確答案寫在答案紙上。

Q.1.5. (5 points)

Suppose the average molecular weight of nucleotides is 330 daltons. Excluding intron and stop codon, what is the mass ratio of dsDNA encoding the 33-kDa protein to the 33-kDa protein? Write down your answer on the answer sheet.

假設核苷酸的平均分子量爲 330 Da。排除內插子與終止密碼，請問轉錄一個 33 kDa 的蛋白質分子的雙股 DNA (dsDNA) 與蛋白質的質量比爲何？請將正確答案寫在答案紙上。

Q.1.6. (5 points)

Suppose a protein P can bind to a protein Q (MW = 1000 daltons). The binding can be revealed by gel-mobility shift assay. Now 200 pmol of protein P were mixed with various amounts (0 to 500 ng) of protein Q. These mixtures were resolved by 10% (w/v) polyacrylamide gel. Gel was stained by Coomassie blue and is shown in **Figure 3**. Calculate the binding molar ratio of proteins P and Q? Write down your answer on the answer sheet.

假設蛋白質 P 會與蛋白質 Q (分子量接近 1000 Da) 結合。結合測試可以藉由膠體移動轉移分析 (gel-mobility shift assay) 達成。現今有 200 pmol 的蛋白質 P 與不同含量的蛋白質 Q (0 to 500 ng) 混合。這些混合物利用 10% PAGE 進行分離後，以 Coomassie blue 染色後如**圖 3** 所示。請計算蛋白質 P 與蛋白質 Q 的莫耳比值 (molar ratio)。

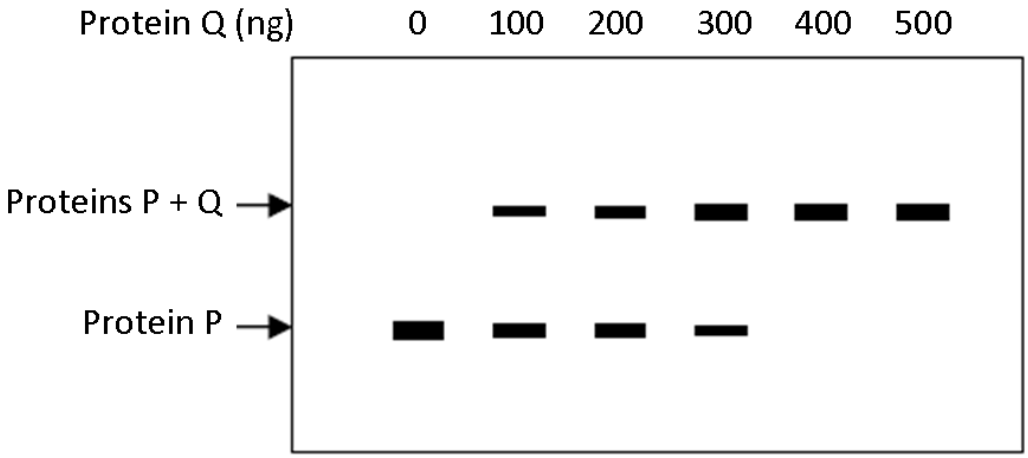

圖 3

Task II (30 Points)

Protein quantification 蛋白質定量

Introduction:簡介

Coomassie Brilliant Blue G-250 (CBG) is a protein staining reagent. It appears in a different color under different pH conditions. It looks reddish brown in acidic solution, whereas it turns blue under neutral or alkaline condition. Since proteins can provide a relative neutral environment, CBG will turn blue with the maximum absorbance at a wavelength of 595 nm when binding to protein. The more protein there is in a sample, the more CBG will bind to it, and thus, the higher intensity of blue color will be. In other words, the absorbance at 595 nm is proportional to the amount of protein in a sample. Based on this, one can determine the concentration of protein by measuring the blue intensity of a sample.

Coomassie Brilliant Blue G-250 (CBG) 是一種蛋白質染劑。在不同的 pH 值下會呈現不同的顏色。酸性溶液中會呈現赭紅色，在中性或鹼性溶液中，則呈現藍色。由於蛋白質大多存在於中性環境下，同時 CBG 會轉變成藍色，當與蛋白質結合後，在 595 nm 下會出現最大吸收值。樣本中，蛋白質含量越多，CBG 結合的越多，同時藍色顏色的強度愈強。換言之，595 nm 吸收值會與蛋白質含量成比例。基於此原理，可以利用藍色顏色的強度來測量蛋白質濃度。

In the problem set, you will perform the following experiment:

依序操作下列實驗並回答問題

1. To make BSA concentration standards (**Table 1**), add 0, 2, 4, 6, 8 and 10 μL of 0.5 mg/mL BSA (green color) in A1 to A6 wells of a microplate (**Figure 4**). Make duplicated BSA concentration standards in B1 to B6 wells. If this step is incorrect, you can repeat the procedure in wells A7 to A12 and/or B7 to B12. Adjust the total volume of each BSA solution to 10 μL by adding an appropriate volume of H_2O (**Table 1**).

   製作 BSA 濃度標準液 (如**表 1**)，如**圖 4** 所示，在盤中的 A1 到 A6 的 well 中分別依序添加 0, 2, 4, 6, 8 and 10 μL 濃度爲 0.5 mg/mL 的 BSA (綠色試管黃色標籤)。重複上

述實驗於盤中的 B1 到 B6 的 well 中。

如果不幸操作錯誤，可以在盤中的 A7 到 A12 well 中與 B7 到 B12 well 中重複此步驟。

最後，分別利用蒸餾水調整每個 well 的 BSA 溶液體積，讓總體積爲 10 μL。

表 1

Materials 材料	**Well of a microplate 微孔盤編號**					
	A1 & B1	**A2 & B2**	**A3 & B3**	**A4 & B4**	**A5 & B5**	**A6 & B6**
0.5 mg/mL BSA (μL)	0	2	4	6	8	10
H_2O (μL)	10	8	6	4	2	0
Diluted BSA concentration (mg/mL) 稀釋後 BSA 濃度	0					

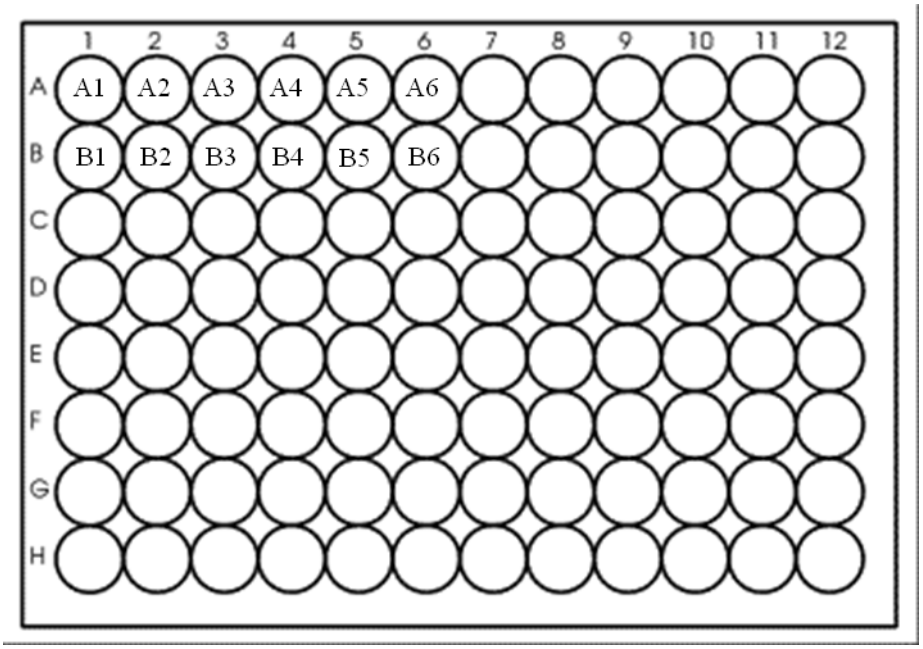

圖 4

2. Add 200 μL of CBG reagent per well in A1 to A6 & B1 to B6. Mix and observe the color change.

   在盤中 A1 to A6 與 B1 to B6 的 well 分別添加 200 μL 體積的 CBG 試劑。混合後並觀察顏色變化。

3. To determine the two concentrations X and Y of enzyme E, add various amounts (2, 4, 6, 8 and 10 μL) of enzyme E (green color) in duplicate to empty wells and bring up the volume to 10 μL with H_2O.

   爲了測量酵素 E 的兩種未知濃度，分別標記爲 X 與 Y。在微孔盤中空的 well 處進行以下實驗。依序添加 0, 2, 4, 6, 8 and 10 μL 的酵素 E 未知濃度 X 與 Y。並用蒸餾水調整每個 well 體積，讓總體積爲 10 μL。重複上述實驗。

4. Add 200 μL of CBG reagent per well to the diluted enzyme E. Mix and observe the color change.

   在步驟 3 的 well 中分別添加 200 μL 體積的 CBG 試劑。混合後並觀察顏色變化。

5. **Lift the sign**, lab assistants will accompany you to measure the absorbance values of your samples at 595 nm using spectrophotometer. Put your Student Code on the print-out data with marker pen.

   完成上述步驟後，**請舉牌**。助教會協助你完成使用分光光度計在 595 nm 下的吸光值測量。用簽字筆在印出的結果上書寫你的考生編號。

6. Return to your work bench, and put the result on the answer sheet using double-sticker.

   回到你的座位上，將結果利用雙面膠帶貼在答案紙上。

Answer the following questions: 回答下列問題

Q.2.1. (10 points)

Calculate the concentrations of BSA in each sample (10 μL) and fill in the blanks in the table on the answer sheet (Q.2.1.1. 5points). Use these values to plot a standard curve of BSA concentrations (X-axis) versus mean absorbance values of duplicated standards (Y-axis) on the answer sheet (Q.2.1.2. 5points).

計算每一個 BSA 標準液中的濃度，並填入答案紙上的表中。(Q.2.1.1. 5 分)

利用所計算出來的蛋白質標準液濃度 (X 軸) 與兩次吸光值結果的平均值 (Y 軸) 在答案紙上繪出標準曲線圖。(Q.2.1.2. 5 分)

Q.2.2. (12 points)

Choose the best sample solution of diluted solution X and Y within the range of BSA standard curve and fill in the table on the answer sheet.

挑選最佳的 X 與 Y 的稀釋倍率，並將結果落在 BSA 濃度標準液範圍內的濃度，分別填入答案紙中的表裡。

Q.2.3. (8 points) Based on the best sample solution you chose, calculate the original concentrations (X and Y) of enzyme E from the standard curve of BSA concentration. The concentrations should be expressed in units of mg/mL. Write down your answers on the answer sheet.

根據你所選出的最佳樣本稀釋溶液，根據上述的標準曲線圖，推算酵素 E 的未知濃度溶液 X 與 Y 的原始濃度。蛋白質濃度單位爲 mg/mL。並將答案填入答案紙中。

Task III (35 points)

Protein purification

Introduction:

Column chromatography is commonly used for purification of proteins. The column is made by packing solid porous material (stationary phase) in a column filled with buffer solution (mobile phase). The protein solution to be separated is loaded on top of the column and allowed to percolate into the solid matrix (stationary phase). A reservoir at the top supplies elution buffer constantly which flows through the matrix and passes out of the column at the bottom (the eluent). Since proteins interact with solid matrix in different degree, individual proteins migrate faster or more slowly through the column depending on their properties. Therefore, one can obtain purified proteins by collecting eluent at different times (**Figure 5**).

簡介

管柱色層分析常用在蛋白質純化的應用，管柱通常分爲兩種相，一種爲填充固態有孔物質(稱爲固定相)，管柱中裝滿緩衝溶液 (稱爲移動相)。待分離的蛋白質溶液將會添加在管柱頂端，並讓其藉由過濾進入固態基質 (即固定相) 中。在管柱的頂端會有一個稱爲貯存器(reservoir) 的構造，其功能是可以保存洗脫緩衝溶液 (elution buffer) 以便通過基質到達管柱底端，此時收集的溶液稱爲洗脫液 (elution)。當蛋白質與基質產生不同程度的交互作用後，蛋白質會因爲物質特性不同而產生快慢不一的移動速度。因此，在不同時間下，每種洗脫液可以純化一種蛋白質 (圖 5)。

Ion-exchange chromatography can be used to separate proteins with different electric charge at a given pH. In anion exchange chromatography, negatively charged proteins bind to positively charged stationary phase. Using solution containing anions to compete with proteins for the adsorption of solid matrix, the bound proteins will be eluted. In practical, proteins are eluted first with buffer containing lower concentration of anion, then with buffer containing higher concentration of anion. Since different charged proteins interact with the stationary phase in different strength, they can be separately eluted by different concentrations of anionic buffers.

離子交換色層分析術的原理是利用在特定的 pH 值下，欲分離的蛋白質會有其各自的電荷，而將其分離。陽離子交換色層分析中，帶負電的蛋白質會被吸附在帶正電的固定相上。

當帶有陽離子的溶液通過管柱時。此時，陽離子會與固定相競爭被吸附的蛋白質，因此蛋白質就會被洗脫出來。實驗進行會先用較低濃度的陽離子洗脫緩衝溶液，最後會換成較高濃度的陽離子洗脫緩衝溶液。因此，吸附在固定相上，分別帶有不同強度電荷的的蛋白質，就會依序被不同強度的陽離子緩衝溶液洗脫出。

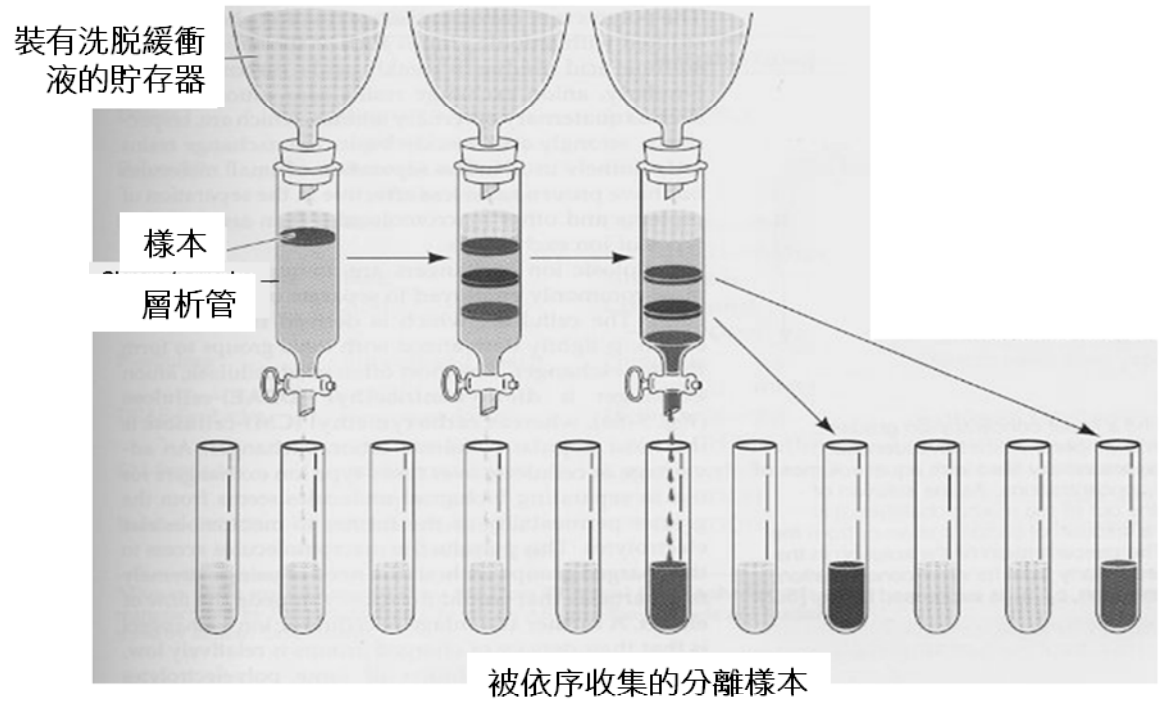

圖 5

In the problem set, you will perform the following experiment (5 points):

依序操作下列實驗並回答問題 **(5 分)**

1. Label six 15-mL centrifuge tubes (yellow cap) a1 to a3 and b1 to b3 accordingly, with a marker pen.

   取 6 支黃色蓋子的 15 ml 離心管，分別以奇異筆標誌 a1, a2, a3, b1, b2, b3。

2. Take the anion chromatography column (**Figure 6A**), un-plug the tube and allow the solution to be drained by gravity in the same centrifuge tube. Plug the tube intermediately when the liquid surface reaches the top of the disc (**Figure 6A**, white arrow). Do not over-dry the gel as it may affect protein purification.

   取一支陽離子交換色層分析管 (如圖 6A)，打開管柱下方栓蓋，利用地心引力的作用，讓管柱內的溶液滴出。當液面到達管柱內碟形物 (disk; 圖 6A，白色箭號處) 的表面時，立刻將原來的栓蓋 蓋上。如果膠體乾燥後，將會影響蛋白質的純化結果。

3. Withdraw 200 μL of protein solution from microcentrifuge tube C (blue tube with blue label) using a P200 micropipette, apply the sample to the chromatography column slowly by touching the filled pipette tip lightly against the inside wall of the tube (**Figure 6B**).

   使用 P200 的微量吸管，自微量離心管 C (藍色標記的藍色管子) 中吸取 200 μL 蛋白質溶液。如圖 6B 所示，將上述蛋白質溶液沿著層析管柱管壁，緩慢注入管中。

4. Un-plug the column and allow the protein sample to drain out, then transfer the column to centrifuge tube a1 (yellow cap). Withdraw 3 mL of anion buffer A (green cap) with a plastic dropper and apply the solution to gel by touching pipette tip against the wall of the tube (**Figure 6C**).

   使用塑膠滴管，自藍蓋離心管中吸取 3 mL 的陽離子緩衝液 A (綠蓋)。旋開栓蓋，讓液體開始滴出。如圖 6C 所示，先將層析管置於 a1 黃色離心管中。再將陽離子緩衝液 A 沿著層析管柱管壁，緩慢注入管中。

5. Collect ~1 mL eluent in centrifuge tubes a1 to a3 (yellow cap) sequentially. It takes about 2 to 3 minutes for each tube.

   分別在標示有 a1, a2, a3 的黃色離心管中，依序收集約 1 mL 的分離洗脫樣本，每管約會耗時 2-3 分鐘。

6. Allow the contents of the column to drain entirely out then transfer the column to centrifuge tube b1 (yellow cap). Withdraw 3 mL of anion buffer B (green cap) with a plastic dropper and apply the solution to gel by touching pipette tip against the wall of the tube (**Figure 6C**).

   讓層析管中的緩衝液流盡。如圖 6C 所示，將層析管置於 b1 黃色離心管中。自另一支藍蓋離心管中吸取 3 mL 的陽離子緩衝液 B (綠蓋)，再將陽離子緩衝液 B 沿著層析管柱管壁，緩慢注入管中。

7. Collect ~1 mL eluent in centrifuge tubes b1 to b3 (yellow cap) sequentially. It takes about 2 to 3 minutes for each tube.

   分別在標示有 b1, b2, b3 的黃色離心管中，依序收集約 1 mL 的分離洗脫樣本，每管約會耗時 2-3 分鐘。

8. Withdraw 50 μL of eluent from tubes a1 to a3 & b1 to b3 (yellow cap) and transfer to centrifuge tubes A1 to A3 & B1 to B3 (red cap), respectively. Mix and observe color change. CBG (see introduction in Task II) reagent in tubes A1 to A3 & B1 to B3 will turn blue when it reacts with the eluted protein.

   分別自標誌 a1, a2, a3, b1, b2, b3 的黃色離心管中吸取 50 μL 的洗脫液，對應加入已添加 CBG (詳見 Task II) 並標誌 A1, A2, A3, B1, B2, B3 紅蓋離心管中，混合均勻，直到有藍色出現爲止。最後觀察並比較顏色變化。

9. After finishing all the experiments, **Lift the sign**, lab assistants will take photo of your experiment results and put a stamp mark on your answer sheet. Without the stamp mark, you will not be evaluated the Q.3.1.1. and Q.3.1.2.

   當時驗完成後，**請舉牌** 助教將會協助你對實驗結果拍照，並在你的答案卷上做戳記。

   如果沒有戳記，Q.3.1.1. 與 Q.3.1.2. 將無法記分。

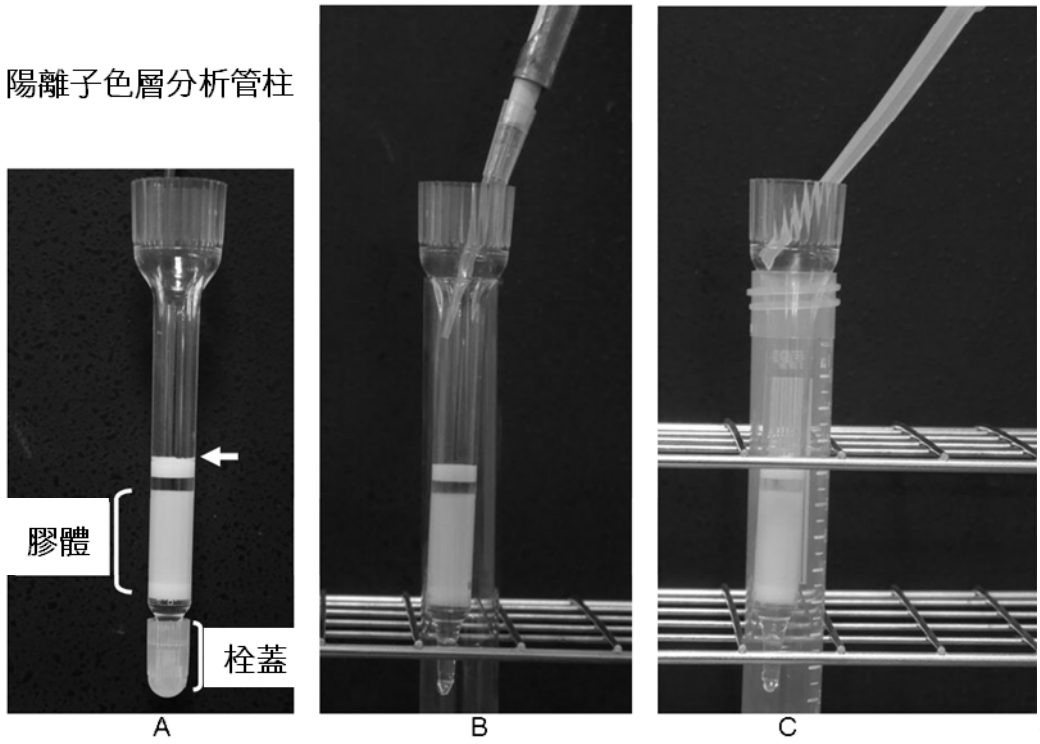

圖 6

Q.3.1. (7 points)

Mark the deepest color change (X) on the answer sheet (Q.3.1.1. 5 points). Which of the following buffers (buffer A or buffer B) can be used to elute the protein? Mark your answer (X) on the answer sheet (Q.3.1.2. 2 points).

找出顏色最深色的離心管，將上面所標示的編號，對應到答案紙 Q.3.1.1. 處，並以 X 標記 (5 分)。

何種陽離子緩衝液 (A 或 B) 可用來洗脫蛋白質？對應到答案紙 Q.3.1.2. 處，並以 X 標記 (2 分)。

Q.3.2. (5 points)

Enzyme A is a protein whose surface is evenly distributed with electric charges. If enzyme A can be eluted from anionic exchange chromatography by high concentration of anionic buffer, what is the property of enzyme A with respect to electric charge? Mark (X) the answer on the answer sheet.

酵素 A 是一種表面佈滿電荷的蛋白質。假設，酵素 A 能被高濃度的陽離子洗脫緩衝溶液純化出來。試問，下列有關酵素 A 電荷的敘述，何者正確？請在正確的答案處以 X 標記。

(A) High negative net charges　高負電荷

(B) Low negative net charges　低負電荷

(C) Zero net charge　零電荷

(D) Low positive net charges　低正電荷

(E) High positive net charges　高正電荷

Q.3.3. (4 points)

Different amino acids differ in the chemical nature of the R group (side chain). **Figure 7** shows four amino acids A, B, C, and D in their prevailing ionic forms at pH 7.2, with the side chain marked in white box. Which of the following amino acids in **Figure 7** would be present more frequently on enzyme A? Write down your answer (X) on the answer sheet.

胺基酸的化學特性來自 R 基 (側鏈)。圖 7 中有四種胺基酸，圖中 R 基以反白表示。R 基所帶電荷情形爲該分子於 pH 7.2 的結果。圖 7 中何種胺基酸的電荷特性最像酵素 A？請在正確的答案處以 X 標記。

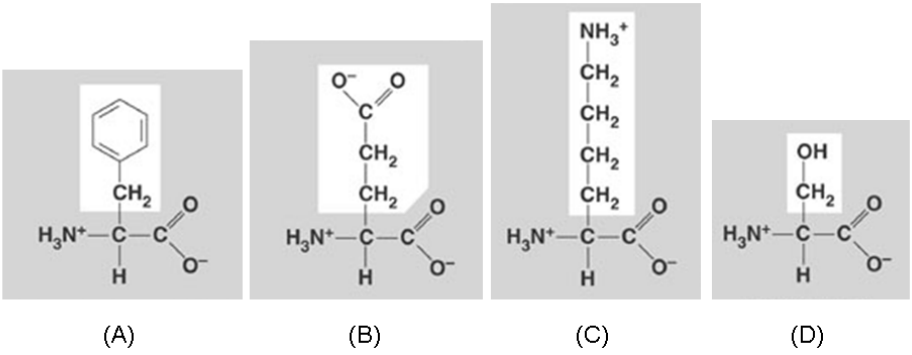

Figure 7

Q.3.4. (5 points)

Hydrophobic interaction chromatography can be used to separate proteins based on their hydrophobicity. To perform the chromatography, protein samples were first treated with buffer containing high concentration of salts such as ammonium sulfate $(NH_4)_2SO_4$, which will remove water molecules from the protein surface. This causes the hydrophobic area on the surface of the protein to be exposed. When the salt-treated proteins are subjected for chromatography, they will be absorbed on the stationary phase through hydrophobic interactions. The higher the hydrophobicity of the protein, the stronger the absorption. As salt concentration can affect the hydrophobic interaction between the protein and the stationary phase, different proteins can be separately eluted by using different concentrations of salt-containing buffers. If enzyme A is highly hydrophobic, which of the following buffers should be used to separate enzyme A from other proteins by chromatography? Mark (X) the answer on the answer sheet.

疏水性作用力色層分析 (Hydrophobic interaction chromatography) 可以是利用蛋白質疏水性的特性進行蛋白質分離。在進行層析前，蛋白質樣本會先以高濃度的鹽類溶液，例如 硫酸銨『$(NH_4)_2SO_4$』進行處理，目的在於去除蛋白質表面的水分子，此時蛋白質的疏水區域便會裸露出來。層析過程中，此種被高濃度鹽類處理過的蛋白質在通過固定相時，便會跟固定相產生疏水性作用力。疏水性作用力越強者，與固定相的吸附力越強。由於鹽類的濃度會影響蛋白質與固定相間的疏水性作用力。因此，不同濃度的鹽類溶液便可以應用於蛋白質分離。假設，酵素 A 具有強烈的疏水性，下列何種緩衝液可以應用於酵素 A 與其他蛋白質的分離？請在正確的答案處以 X 標記。

(A) Low-salt buffer　低濃度鹽類的緩衝液

(B) High-salt buffer　高濃度鹽類的緩衝液

(C) Buffer without salt　無鹽類的緩衝液

(D) Low-salt buffer first then high-salt buffer

先使用低濃度的鹽類緩衝液再置換成高濃度的鹽類緩衝液

(E) High-salt buffer first then low-salt buffer

先使用高濃度的鹽類緩衝液再置換成低濃度的鹽類緩衝液

Q.3.5. (4 points)

If enzyme A is highly hydrophobic, which of the amino acids in **Figure 7** would be present more frequently on enzyme A? Mark (X) the answer on the answer sheet.

假設，酵素 A 具強烈的疏水性，圖 7 中何種胺基酸在酵素 A 中比例最高？請在正確的答案處以 X 標記。

Q.3.6. (5 points)

Gel filtration chromatography separates proteins based on their sizes. The gel, or stationary phase, consists of cross-linked polymer beads with engineered pores of a particular size. Small proteins enter the pores and are retarded by their more labyrinthine path. Large proteins cannot enter the pores and so take a short path through the column, around the beads. **Table 2** is a list of gels and their fractionation ranges. Suppose both enzyme A (22 kDa) and protein B (44 kDa) are single-subunit proteins. One would like to purify enzyme A from a mixture containing enzyme A and protein B using gel filtration chromatography. Which gel is best suited for the job? Mark your answer (X) on the answer sheet.

膠體過濾色層分析 (Gel filtration chromatography) 的原理是利用蛋白質的分子大小達到分離的效果。固定相 (膠體) 是利用能以鏈結模式而產生許多不同孔徑大小的聚合物所構成。小分子的蛋白質必須經過較長的路徑才能離開膠體，因此較爲費時。由於大分子蛋白質無法穿過小孔徑，相對地通過膠體的時間較短。表 2 列出爲各式膠體所適用分離蛋白質分子大小的範圍。酵素 A (分子量爲 22kDa) 與蛋白質 B (分子量爲 44kDa) 均爲單元體蛋白 (single-subunit protein)。今天想要將自含有酵素 A 與 蛋白質 B 的混和液中，利用膠體過濾色層分析法將酵素 A 分離出來。請問下列何種固定相最適宜進行實驗？請在正確的答案處以 X 標記。

Table 2

Types of stationary phase 固定相種類	Fractionation range (MW, Da) 分離蛋白質分子大小的範圍 (分子量單位 Da)
G-10	<700
G-15	<1500
G-25	1,000-6,000
G-50	1,500-30,000
G-75	3,000-70,000
G-100	4,000-150,000
G-150	5,000-400,000
G-200	5,000-800,000

Q.3.7. (5 points)

Assume that the concentration of total proteins in the original solution is 1 mg/mL and the activity of enzyme A is 0.5 units in 1-mL protein sample. The concentration of total proteins after purification is 0.1 mg/mL and the activity of enzyme A is 1 unit in 1-mL protein sample. Calculate the purification factor (times of purity improvement) of enzyme A. Write down your answer on the answer sheet.

假設，原始樣本總蛋白質濃度爲 1 mg/mL，酵素 A 活性爲 0.5 單位 / mL。酵素 A 經過純化並濃縮後，總蛋白質濃度爲 0.1 mg/mL，酵素 A 活性爲 1 單位 / mL。請計算酵素 A 純化因子 (purification factor)「純度增進倍數」，並將正確答案寫在答案紙上。