Biochemistry 生物化學

34th International Biology Olympiad

3-10 July 2023, United Arab Emirates University

第 34 屆國際生物奧林匹亞競賽

2023-7-3 到 2023-7-10 在 UAE 大學

Practical Exam

Biochemistry

Total points: 100

Duration: 90 minutes

生物化學實驗考試

總分：100 分 考試時間：90 分鐘

General Instructions:

You have 90 minutes to complete **ONE task in this practical exam, plus 9 questions.**

Task: Purification, quantitative analysis and kinetic measurements of bacterial diaphorase activity (100 points)

Important information:

Write your answers in the answer sheet. Only answers given in the answer sheet will be evaluated.

Make sure that you have received all the materials and equipment listed, including a graph paper. Check tubes contain solutions (with exception of tubes 1 to 12). If any of these items are missing, please raise your card immediately.

Read the protocol before you start.

During experiments, ensure that you wear gloves, and handle the equipment and samples carefully. Compounds A and B are hazardous.

Any spilled solutions, samples or equipment damaged by you will not be replaced, but the gloves can be replaced.

Your plate results will be scored and you do not need it anymore.

Use the following cards to ask for water/washroom/help.

一般指示：

你有 90 分鐘的時間完成本實驗考試中的 **1 個任務，加上 9 個問題。**

任務：細菌氫傳遞酶的純化、定量分析以及酵素活性動力學測量（100 分）

重要資訊：

將答案寫在答題卡上。只有在答題卡上填寫的答案才會被計分。

確保你有收到所有列出的材料和設備，包括一張圖紙。如果有任何缺失的物品，請立即舉牌。檢查試管內是否有液體 (除了管 1 到管 12)，如果有任何東西缺漏，請立刻舉牌。

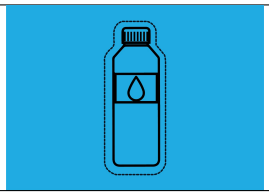
實驗開始前請務必閱讀實驗步驟

在實驗過程中，請確保你有戴上手套，並小心處理設備和樣品。化合物 A 與 B 是有毒的。

任何人為造成的溶液、樣品溢出或設備的損壞都將無法更換。

你的 96 孔盤的讀值將用以評分，但讀完後即不再需要

需要請求水/洗手間/幫助時，請使用以下的卡片

Drinking water 飲用水	Washroom 洗手間	分光光度計測量	Other queries 其他請求
			

Stop answering as soon as you hear the whistle at the end of the exam.

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

Good luck!

當你聽到考試終止的哨聲時，請立刻停止作答

任何紙張、材料或是設備都不可以帶離實驗室

祝好運！

Required materials with labels and equipment:

1. Eppendorf tubes labelled with the numbers from 1 to 12
2. 1.0 ml of bacterial lysate (BL)
3. Standard BSA solution (BSA)
4. Lysis buffer (LB)
5. Wash buffer (WB)
6. Elution buffer (EB)
7. Bradford buffer (BB)
8. Substrate solution (SUB)
9. Compound A (A)
10. Compound B (B)
11. Assay buffer (AB)
12. Bradford reagent (BR) in a black 15 ml tube in the paper cup
13. 96-well plate
14. Affinity chromatography column with resin, on the column holder
15. 10-100 µl Micropipette
16. 100-1000 µl Micropipette

17. Yellow tips for 10-100 µl Micropipette
18. Blue tips for 100-1000 µl Micropipette
19. Paper cup to discard waste
20. Piece of aluminium foil
21. Piece of parafilm
22. Pair of gloves
23. Paper white cup for discarding liquids under the column

所需的材料與設備應以標記，請確實檢查：

1. 標有 1 至 12 的 Eppendorf 微量離心管
2. 1.0 ml 細菌裂解液（BL）
3. BSA 標準溶液（BSA）
4. 裂解緩衝液 (LB)
5. 洗滌緩衝液（WB）
6. 洗脫緩衝液（EB）
7. Bradford 布拉德福德緩衝液（BB）
8. 受質溶液（SUB）
9. 化合物 A (A)
10. 化合物 B(B)
11. 測定緩衝液 (AB)
12. 一個紙杯內含黑色 15 ml 離心管，其中裝有 Bradford 布拉德福德試劑（BR）
13. 96 孔盤
14. 帶有樹脂的親和層析管柱，放在管柱架上
15. 10-100 µl 微量吸管
16. 100-1000 µl 微量吸管
17. 適用於 10-100 µl 微量吸管的黃色 tip
18. 適用於 100-1000 µl 微量吸管的藍色 tip
19. 丟棄廢液的紙杯
20. 一塊鋁箔
21. 一塊石蠟封口膜
22. 一副手套
23. 一個用來接收層析管柱流出廢液之白色紙杯

Introduction:

Diaphorase (NADH dehydrogenase) is an enzyme (23.0 kDa) that converts nicotinamide adenine dinucleotide (NAD) from its reduced form (NADH) to its oxidized form (NAD+):

NADH + H+ + acceptor $\rightleftharpoons$ NAD+ + reduced acceptor

Diaphorase is a flavoprotein that contains iron-sulfur centers. It is used in the electron transport chain for ATP generation.

In this experiment, you will purify a bacterial diaphorase from a crude extract, then measure its activity.

First, you must purify diaphorase from a crude bacterial lysate. *E. coli* has previously been transformed with a plasmid expression vector, into which the gene encoding diaphorase tagged with 6-histidine had been cloned. You will purify diaphorase by affinity chromatography; the 6-histidine tag has affinity for, and binds to, nickel which is attached to the resin in the (Ni-NTA Sepharose) columns.

The bound protein can be eluted from the resin by changes of buffers used in the chromatography protocol. You will collect eluted fractions into two Eppendorf tubes.

After protein elution, you will determine the protein concentration of the two fractions, using a Bradford quantification assay. The Bradford assay is a colorimetric assay during which binding of ‘Coomassie brilliant blue’ to protein results in increased absorbance of 595 nm wavelength light. You can determine the protein concentration of your fractions by comparing their absorbance to a standard curve derived by assaying bovine serum albumin (BSA) protein solutions of known concentration.

Finally, you will measure the activity of diaphorase in one of the fractions using a colorimetric assay in the absence and presence of some additives. The reduced form of the acceptor (NBT) has a maximum absorbance at the same wavelength of Bradford reagent (595 nm).

介紹：

氫傳遞酶（NADH 脫氫酶）是一種酵素 (23.0 kDa)，可將菸鹼醯胺腺嘌呤二核苷酸 (NAD) 從還原形式 (NADH) 轉化為氧化形式 (NAD+)：

NADH + H+ + 氫接受體 ⇌ NAD+ + 還原態氫接受體。

氫傳遞酶是一種含有鐵硫中心的黃素蛋白。它在電子傳輸鏈中用於 ATP 生成。

在本實驗中，你將從粗提取液中純化細菌氫傳遞酶，然後測量其活性。

首先，你必須從細菌粗提取液中純化氫傳遞酶。先前大腸桿菌已被帶有 6-組胺酸標籤 (tag) 的氫傳遞酶基因的載體轉殖，並大量表現。你將使用親和性管柱層析法來純化氫傳遞酶。6-組胺酸標籤對於管柱中的 Ni-NTA Sepharose 樹脂上的鎳具有親和力，可與其結合，並附著於管柱中。

透過改變層析過程中所使用的緩衝液，可以洗脫出結合在樹脂上的蛋白質。請將洗脫出來的液體分液 (fraction) 收集到兩個微量離心管 (Eppendorf) 中。

蛋白質洗脫後，請用 Bradford 定量法測定兩個分液中的蛋白質濃度。Bradford 定量法是一種比色測定，是利用' 考馬斯亮藍 Coomassie blue' 與蛋白質結合後，會增加 595 nm 的吸光度。你可以將從層析管柱所洗脫出之分液產生的吸光值與量測已知蛋白質濃度的牛血清白蛋白 (BSA) 溶液所得的標準曲線進行比較。這樣就可以確定不同洗脫液分液的蛋白質濃度。

最後，你將使用比色測定法。在某些添加劑的添加和不添加的情況下，量測洗脫液其中一個分液中的氫傳遞酶活性。請注意，還原態的氫接受體 (NBT) 跟 Bradford 試劑同樣在波長 595 nm 有最大吸收值。

Part 1. 第一部分

Purification of bacterial diaphorase using Ni-NTA affinity chromatography.

Procedure:

In all steps, take care to avoid the resin **drying out.**

The chromatography column is already placed in the hole of the column holder rack. Note that it fits into the hole tightly. Handle the column carefully during the course of the experiment. The column is sealed at the bottom with a stopper and the resin in the column is covered with a small volume of 20% ethanol.

It is important you perform the following steps quickly and carefully to protect the resin.

1. Carefully look at the contents of the column to identify the border between the resin and the overlaying liquid.
2. Break and remove the stopper at the bottom of the column by twisting it clockwise and anticlockwise.
3. Unscrew the lid of the column to allow the liquid to start flowing through the resin. Discard the flow-through into the paper cup placed under the column stand. Collection should continue just until there is no liquid left above the resin. Approximately **5 - 7 drops** will be discarded.
4. Gently add **500 µl** of lysis buffer (LB) to the column, without disrupting the resin, in order to equilibrate the column with lysis buffer. Discard the flow-through into the paper cup placed under the column stand. Collection should continue just until there is no liquid left above the resin.
5. Add **500 µl** of provided bacterial lysate (BL) onto the column and discard the flow-through into the paper cup placed under the column stand. Be sure that all the bacterial lysate has entered the column. Collection should continue just until there is no liquid left above the resin.
6. **Wash Step**: Add 500 µl wash buffer (WB) and discard the flow-through into the paper cup placed under the column stand. As the volume of the buffer above the resin decreases to about 50 µl, add 500 µl additional wash buffer and continue collection of flow-through into the same cup. Add a further 500 µl to wash a third time and continue collecting into the same cup. Collection should continue just until there is no liquid left above the resin.
7. **Elution Step:** Gently transfer the column to the Eppendorf **tube 1**, in the rack or in the column holder. Add **500 µl** elution buffer (EB) to start release of protein (diaphorases) bound to the resin in the column. Start collecting drops into **tube 1**. As the volume of the buffer above the resin decreases to about **50 µl**, add **500 µl** additional elution buffer and continue collection of the eluent into **tube 2**. (Approximately 1000 µl will be collected, into tubes 1 and 2 in total).
8. Seal the bottom of the column with a provided piece of parafilm and place the screw cap on top.

NOTE: Tube 3 will not be used.

使用 Ni-NTA 親和層析法純化細菌氫傳遞酶。

實驗步驟：

在所有步驟中，請注意避免樹脂**乾掉**。

層析管柱已放置在管柱架的孔中。注意，管柱是可以緊密貼合孔洞的。在實驗過程中請小心操作層析管柱。管柱底部有一個塞子將管柱密封，而管柱內的樹脂則是用少量 20% 乙醇浸泡覆蓋。

請快速並小心執行以下步驟，這對於保護管柱內的樹脂非常重要:

1. 仔細查看管柱內容物，以便清楚識別管柱內樹脂與浸泡液體間的邊界。

2. 以順時針方向或逆時針方向旋轉，折斷並移除管柱底部的塞子

3. 擰開管柱上蓋，讓液體開始流過樹脂。丟棄剛開始的液體，利用管柱下方的紙杯。持續直到樹脂中無液體殘留 (丟棄的液體大約 **5-7 滴**)。

4. 輕輕向管柱中添加 **500 µl** 裂解緩衝液 (LB)，小心操作，不要破壞樹脂表層平整。這是為了使管柱中的樹脂與裂解緩衝液達成平衡。利用管柱下方紙杯，接收流出的廢棄液，持續收集，直到樹脂中無液體殘留。

5. 將所提供的 **500 µl** 細菌裂解液 (BL) 添加到管柱中，確保所有細菌裂解液已倒入管柱。用管柱下方紙杯，接收流出的廢棄液。持續收集，直到樹脂中無液體殘留。

6. **洗滌步驟：**添加 500 µl 洗滌緩衝液 (WB)，利用下方紙杯接收流出的廢棄液。隨著樹脂上方緩衝液的體積減少至約 50 µl，再添加 500 µl 額外的洗滌緩衝液，並繼續接收廢棄液到紙杯中。最後，再取 500 µl 進行第三次清洗，並繼續接收廢棄液到紙杯中。持續收集，直到樹脂中無液體殘留。

7. **洗脫步驟：**將管柱輕輕置放在微量離心管架或管柱支架上的微量離心管 1 號 (**管 1**) 之上。添加 **500 µl** 洗脫緩衝液 (EB)，開始釋放附著於柱內樹脂中的蛋白質 (氫傳遞酶)。開始收集管柱中流出來的液體到**管 1** 中。當管柱的緩衝液體積減少至約 **50 µl** 時，再添加 **500 µl** 額外的洗脫緩衝液，並同時使用**管 2** 繼續收集洗脫液（總共大約 1000 µl 將會被收集到兩個微量離心管中）。

8. 用提供的石蠟封口膜密封層析管柱底部，然後將螺帽鎖於層析柱上。

注意: 不會用到管 3

Part 2. 第二部分

Measurement of diaphorase **protein** using a quantitative method

使用定量方法測定氫傳遞酶**蛋白**

Q.2.1	Complete the protocol below. The Bradford assay will worth 33 points. 完成下列實驗步驟，其中 Bradford assay 佔 33 分	33.0pt

1. Make bovine serum albumin (BSA) dilutions as shown in Table 1:

1. 如表 1 指示製作牛血清白蛋白 (BSA) 稀釋液：

Tube number 管號	4	5	6	7	8	9	10
Standard BSA (1 mg/ml), µl BSA 標準品, 1 (mg/ml), µl	0	20	40	60	80	100	120
Bradford buffer, µl Bradford 緩衝液, µl	200	180	160	140	120	100	80

2. Ensure the contents of each tube (4-10) are well mixed by pipetting up and down.

2. 透過微量吸管上下吸放，確保每管 (4-10) 的內容物混合均勻。

3. Make dilutions of tubes 1 and 2 in tubes 11 and 12 as shown in Table 2:

3. 如表 2 所示，分別製作管 1 和 2 的稀釋樣品，到管 11 和 12 中:

	11	12
Eluted protein 洗脫蛋白	50 µl of 1 來自管 1 的 50 µl	50 µl of 2 來自管 2 的 50 µl
Bradford buffer Bradford 緩衝液	50 µl	50 µl

4. Each sample will be assayed in duplicate. For this purpose, add 10 µl of tubes 4-10 into wells of A1-A7, and then consecutively to wells of C1-C7, of your 96 well plate. Subsequently, add 10 µl of tubes 11 into A9 and C9, and 10 µl of tube 12 to wells A11 and C11.

Pause the Bradford assay at this stage, and start Part 3 in the same plate.

4. 每個樣品都將進行兩次測定。為此，請從管 4-10 中分別取 10 µl，添加到 96 孔盤的 A1-A7 孔位中。然後持續同樣次序添加到 C1-C7 孔中。隨後，將管 11 取 10 µl 分別添加到 A9 和 C9 中，將管 12 取 10 µl 分別添加到孔 A11 和 C11 中。

在此階段，先暫停 Bradford 測定工作，並在同一個孔盤中開始第三部分

Part 3. 第三部分

Measurement of diaphorase activity using a colorimetric assay

使用比色測定法測量氫傳遞酶活性

Q.3.1 Complete the protocol below. The enzyme assay will worth 15 points. 15.0pt
完成下列實驗步驟，其中酵素活性測定佔 15 分

Prepare the following solutions in Table 3 into wells E1-E7. Use the enzyme you purified into tube 1 and 2 **without dilution**.

依照表 3 將以下溶液製備到 E1-E7 孔位中。這裡使用的酵素來自管 1 和管 2，直接使用**無需稀釋**。

Table 3 - Enzyme assay

表 3 酵素測定

Well number 管號	E1	E2	E3	E4	E5	E6	E7
Enzyme (μl) - tube 1 酵素 (μl) -管 1	-	20	-	20	20	20	20
Enzyme (μl) - tube 2 酵素 (μl)-管 2	20	-	20	-	-	-	-
Compound A 化合物 A	-	-	-	10	10	-	-
Compound B 化合物 B	-	-	-	-	-	10	10
Assay buffer (AB), μl 測定緩衝液 (AB), μl	190	10	10	-	-	-	-
Substrate (SUB), μl 反應受質 (SUB), μl	-	180	180	180	180	180	180

Immediately continue with the Bradford assay in the same plate.

Bradford Assay (Continued)

5. Add 190 μl of the Bradford reagent (BR) into each of the wells (**only A1-A7, A9, A11 and C1- C7, C9 and C11**) to which samples had been added in the previous stage (do **not** add Bradford reagent to wells E1-E7, used for the enzyme assay). Mix the contents of each well gently with a micropipette tip. Take care not to create bubbles; bubbles could interfere with absorbance measurements.

6. Cover the 96-well plate with aluminium foil to prevent exposure to light. Incubate in the dark for 5 minutes. Keep your time using wall clocks in front of you.

7. After completion of the 5-minute incubation, raise your card (Spectrophotometer image). A scientific volunteer will take your coded plate to a Spectrophotometer station and have the absorbance of all your wells read at a wavelength of 595 nm.

Note: The 96-well plate will <u>not</u> be accepted for reading after the exam is finished.

立即在同一孔盤中繼續進行之前的 Bradford 測定。

Bradford 測定（續）

5. 在前一階段已添加樣品的每個孔位 **(只有 A1-A7、A9、A11 和 C1-C7、C9 和 C11)** 添加 190 µl 的 Bradford 試劑 (BR)。請**不要**在 E1-E7 孔位中添加 Bradford 試劑，這些將用於酵素活性測定。用微量吸管輕輕混合每個孔位的內容物。注意不要產生氣泡，氣泡可能會干擾吸光度測量結果。

6. 用鋁箔覆蓋 96 孔盤以防止光照。在黑暗中反應 5 分鐘。使用牆上的鐘來計時。

7. 在反應 5 分鐘後，舉起標有分光光度計之卡片 (Spectrophotometer image) ，此時助教會將帶著有你號碼的孔盤到分光光度計測量站，並在 595 nm 波長下讀取所有孔位的吸光度。

注意：在考試結束後，就<u>無法</u> 要求進行 96 孔盤測量。

Part 4 第四部分

The experiments in the previous sections were previously carried out by other students. You are given the absorbance readings at 595 nm in the table below. Calculate the mean of absorbance and BSA concentration (tubes 4-10) and write your answers to 2 decimal places. Use this data to answer the questions in Part 4.

有其他同學先前已經做過前面幾節的實驗，他們的 595 nm 吸光度讀數紀錄在下表中。請計算吸光度平均值和 BSA 濃度（管 4-10），並寫下到小數點後兩位的答案。繼續使用此數據來回答第四部分中的問題。

Q.4.1 4.0pt

BSA (mg/ml)	Well 孔位	A (595 nm)	Well 孔位	A (595 nm)	Mean 平均值
	A1	0.50	C1	0.40	
	A2	0.60	C2	0.50	
	A3	0.74	C3	0.62	
	A4	0.84	C4	0.80	
	A5	0.95	C5	0.91	
	A6	1.00	C6	0.96	
	A7	1.10	C7	0.99	
Tube 11 管 11	A9	0.90	C9	0.94	
Tube 12 管 12	A11	0.58	C11	0.56	

Q.4.2 Based on the linear part of data, presented in the table above, draw a calibration curve between BSA concentration, mg/ml (A) and mean absorbance at 595 nm (B). You should indicate all of your points in the graph paper. Label the axes of the graph using the A and B letters and indicate corresponding concentration of tubes 11 and 12.
根據上表中線性部分的數據，繪製 (A) BSA 濃度 mg/ml 和 (B) 595 nm 平均吸光值之間的校準曲線。你應該在圖表上標示出所有的點。使用字母 A 與 B 標記圖表的兩軸，並指示出管 11 和 12 的相應濃度。 15.0pt

Q.4.3 Using the standard curve and the absorbance at 595 nm of samples from tubes 11 and 12, calculate the protein concentration of the fractions in tubes 1 and 2. Write the protein concentrations of tubes 1 and 2 (rounded to two decimal places) on the answer sheet. Molecular weight of diaphorase is 23.0 kDa.
使用這個標準曲線，以及從管 11 和 12 樣品在 595 nm 讀取的吸光值，計算管 1 和 2 洗脫分液中的蛋白質濃度。
在答題紙上寫下管 1 和 2 的蛋白質濃度 (四捨五入到小數點後兩位)。氫傳遞酶的分子量為 23.0 kDa。 10.0pt

Tube Number 管號	Mean Absorbance (595 nm) from table at beginning of Q4.1 平均吸收值 (595 nm) 從 Q4.1 開始時的表格	Dilution Factor 稀釋倍數	Tube Number 管號	Concentration (mg/ml) 濃度 (mg/ml)	Concentration (μM)
11			1		
12			2		

Kinetic parameters calculation

The same students measured the enzyme activity of 10 µl from **tube 2** in a total volume of 1.0 ml. Absorbance of the solution was measured over a 1-cm path length.

Over 3 minutes, the absorbance at 595 nm increased linearly by a total of 0.6 units.

Assume the extinction coefficient of NBT to be 12.3 $M^{-1}\,cm^{-1}$. What was the specific activity of the enzyme purified by this student? Write the answer to 2 decimal places, in Table Q.4.4

動力學參數計算

同一批學生的實驗，從總體積 1.0 ml 的**管 2**，測量其中 10 µl 的酵素活性。使用 1 cm 光徑長度來測量溶液的吸光度。

在 3 分鐘後，595 nm 的吸光值，總計會線性增加 0.6 個吸收單位。

假設 NBT 的消光係數為 12.3 $M^{-1}\,cm^{-1}$。這群學生純化出來的酵素比活性是多少？請在表 Q.4.4 內填入到小數點後兩位的答案。

Q.4.4 5.0pt

Specific activity 比活性 (U/mg) **or µmoles** $\times$ **min**$^{-1}$ $\times$ **mg**$^{-1}$	

As a rule, enzyme kinetics, can be described by the Michaelis-Menten equation:

$V_0 = \frac{V_{max}[S]}{K_m + [S]}$

where,

V_0 is reaction rate,

V_{max} is maximal reaction rate.

S is substrate concentration

K_m is Michaelis-Menten constant.

When the oxidised NBT (substrate) concentration is 20 μM, diaphorase activity is 6500 μmoles $\times$ min^{-1} $\times$ mg^{-1}.

K_m is equal to 10 μM.

$k_{cat} = \frac{V_{\text{max}}}{[E]}$

where,

k_{cat} is enzyme turn over number and [E] is the concentration of the enzyme.

依照規則，酵素動力學可以依照 Michaelis-Menten 方程式來描述:

$V_0 = \frac{V_{max}[S]}{K_m + [S]}$

此處，

V_0 是反應速率

V_{max} 是最大反應速率

S 是受質濃度

K_m 是 Michaelis-Menten 常數

當氧化態的 NBT (受質) 濃度為 20μM，氫傳遞酶活性是 6500 μmoles $\times$ min^{-1} $\times$ mg^{-1}

K_m 等於 10 μM

$k_{cat} = \frac{V_{\text{max}}}{[E]}$

此處，

k_{cat} 是酵素轉換率，而 [E] 是酵素的濃度

Q.4.5 Calculate the V_{max} and k_{cat} for the diaphorase. Write the answer to 2 decimal places. 10.0pt
計算氫傳遞酶的 V_{max} 跟 k_{cat} 。填入到小數點後兩位的答案。

V_{max}	
$k_{cat}\ (sec^{-1})$	

Q.5

Conventional purification

To purify two proteins, A (MW: 25 kDa, pI: 4.5) and B (MW: 55 kDa, pI: 8.8) from a crude extract, based on their physical properties, a size exclusion chromatography (using appropriate Sephadex type) and an anion exchange chromatography (DEAE-Sepharose) column were used.

Identify the following statements as True or False by marking a cross (X) in the appropriate cell

傳統純化

粗提取液中有蛋白 A (分子量 25 kDa，等電點 pI:4.5) 與蛋白 B (分子量 55 kDa，等電點 pI:8.8)。要依照它們的物理特性，使用一個分子篩層析管柱 (使用適合的 Sephadex 樹脂) 以及一個陰離子交換層析管柱 (DEAE-Sepharose)，來純化它們。

判斷下面的描述是正確 (True) 或是錯誤 (False)，在適當格子中打一個叉叉)

Q.5.1 4.0pt

Statement 描述	True 正確	False 錯誤
Protein A has a higher affinity to DEAE-Sepharose than B at pH 7.0. 蛋白 A 相較於蛋白 B，在 pH 7.0 時對於 DEAE-Sepharose 有較強的的親和力		
Protein A elutes faster than B from Sephadex. 蛋白 A 在相較於蛋白 B，在 Sephadex 洗脫的速度較快		
Protein A elutes faster than B from a cation exchange resin at pH 4.5. 在 pH 4.5 時，蛋白 A 相較於蛋白 B，在陽離子交換樹脂中洗脫的速度較快		
Protein A elutes from DEAE-Sepharose at higher salt concentration than protein B at pH 7.0 在 pH7.0 時，蛋白 A 相較於蛋白 B，在高鹽環境下從 DEAE-Sepharose 洗脫的速度較快		

Q.5.2

Q.5.2 **Bacterial hosts** 細菌宿主 4.0 分 4.0pt

Different *E. coli* strains are optimized for expressing different kinds of synthetic proteins, as shown in the table below.
如下表所示，不同的大腸桿菌菌株經過優化，用來表達不同種類的合成蛋白質。

Bacterial Code 細菌代碼	**Bacterial strain (*E. coli*)** 細菌菌株（大腸桿菌）	**Characteristic** 特徵
A	BL21- Codon Plus	For rare codon expression 用來表達稀有密碼子
B	Origami	Improve disulfide bond in cytosol. 改善細胞質中的雙硫鍵形成
C	BL21-Codon Plus (DE3)-RP-X	Enhance expression of heterologous proteins. 增強外源蛋白的表達
D	C43 (DE3)	Prevents cell death due to expression of toxic proteins. 防止細胞因表現毒性蛋白而死亡
E	SoluBL21	Suitable for expression of insoluble protein in soluble form 適合可將不可溶蛋白質表現成可溶形式
F	C41 (DE3)	Suitable for expression of membrane proteins 適合表現膜蛋白

Which *E. coli* strain is most suitable for the expression of the following proteins? Choose only one option for each protein, and write the ‘Bacterial code’ from the above table:
請問哪種大腸桿菌菌株最適合用來表達以下蛋白質？每種蛋白質僅可選擇一個細菌選項，並寫下上表中的“細菌代碼”：

Protein 蛋白質	**Bacterial code** 細菌代碼
Insulin 胰島素	
N-terminal domain of Gasdermin D with ability to make a membrane pore Gasdermin D 蛋白的 N 端，其具有形成穿膜孔洞的能力	
Glut 1, which is responsible for glucose transport Glut 1，負責葡萄糖轉運	
Proteins with high beta-sheet content 具有高度 beta 折疊的蛋白質	