Country: _____	Student Code: _____

23rd INTERNATIONAL BIOLOGY OLYMPIAD

8th – 15th July, 2012

SINGAPORE

PRACTICAL TEST 2

MICROBIOLOGY & BIOCHEMISTRY

微生物學&生化學

Total points: **100** 總分：100

Duration: **90 minutes** 時間: 90 分鐘

Dear Participants

- In this test, you have been given the following two tasks:

  本測驗，你有兩個任務：

  Task 1: Bacteriophage: an effective agent in the killing of bacteria. (50 points)

  任務一： 噬菌體：一個有效的媒介用於殺死細菌。（50分）

  Part A: Effects of Phage and antibiotics on the killing of antibiotic-resistant *E. coli* (31 points)

  部分A：噬菌體和抗生素對殺死具抗藥性之大腸桿菌的影響（31分）

  Part B: Phage titre and multiplicity of infection (19 points)

  部分B：噬菌體效價和感染的多樣性（19分）

  Task 2: Titration of an amino acid. (50 points)

  任務二：滴定一個胺基酸（50分）

- Use the **Answer Sheet**, which is provided separately, to answer all the questions.

  使用**答案卷**回答所有問題，答案卷與題目卷是分開的。

- The answers written in the Question Paper will **NOT** be evaluated.

  答案寫在題目卷將**不給分**。

- Write your answers legibly in ink.

  清楚易讀地以原子筆寫下你的答案。

- Please make sure that you have received all the materials and equipment listed for each task.

  If any of these items are missing, please raise your hand **immediately**.

- Stop answering and put down your pen IMMEDIATELY when the bell rings.

- At the end of the test, place the Answer Sheets and Question paper in the envelope provided.

  Our Assistants will collect the envelope from you.

Have fun and Good Luck! ☺

Materials and equipment: 材料和器材

For Task I: Bacteriophage: an effective agent in the killing of bacteria

任務一：噬菌體：一個有效的媒介用於殺死細菌。

Materials and equipment 材料和器材	**Quantity**	**Unit**
micropipette tips 10µl 微量滴管尖	1	Box 盒
micropipette tips 200µl 微量滴管尖	1	Box 盒
micropipette tips 1000µl 微量滴管尖	1	Box 盒
micropipette 1 - 10µl 微量滴管	1	Piece 支
micropipette 2 - 20µl 微量滴管	1	Piece 支
micropipette 20 - 200µl 微量滴管	1	piece 支
micropipette 100 - 1000µl 微量滴管	1	piece 支
microfuge tube rack 微量離心管盒	1	piece 個
cuvette rack 比色管盒	1	piece 個
microfuge tubes (in a beaker) 微量離心管 （放於燒杯內）	Many 很多	tube 管
Stock *E. coli* culture (1×10^7 cells/ml) in LB broth 以LB培養液培養的大腸桿菌原液（濃度 $1x10^7$ 個細胞/毫升）	1	Tube 管
LB broth (in a 50 ml Falcon tube) LB 培養基（放在一個 50 毫升的離心管）	1	Tube 管
sterile deionized water in microfuge tube 滅過菌的去離子水（放在微量離心管中）	1	Tube 管
ampicillin stock (1 mg/ml) dissolved in deionized water 溶解於去離子水的 ampicillin 抗生素原液（1 mg/ml）	1	Tube 管
bacteriophage stock (10^8 pfu/ml) in deionized water 溶於去離子水的噬菌體原液（濃度為 10^8 pfu/ml）	1	Tube 管
cuvettes (in a beaker) 比色管（放在一個燒杯內）	4	Piece 個
Stopwatch 碼錶 計時器	1	Piece 個
floating rack (labelled with your country code) 可漂浮的圓形微量離心管架 （已經標示了你的國碼）	1	piece 個
water-bath 37 ºC (there is one assigned for your usage) 37 ºC 的水浴槽（有指定一個讓你使用）	1	Set 組

UV-VIS Spectrophotometer (there is one assigned for your usage) UV 至可見光的分光光度計（有指定一個讓你使用）	1	Set 組
photographs of *E. coli* plates (A to H) 大腸桿菌培養盤的照片 （標示 A 到 H）	1	Set 組

For Task II: Titration of an amino acid

任務二：任務二：滴定一個胺基酸

Materials and equipment 材料和器材	**Quantity**	**Unit**
25 ml burette 25 ml 有刻度的玻璃滴管	1	Piece 個
25 ml pipette 25 ml 的玻璃吸管	1	Piece 個
100 ml beakers 100 ml 的燒杯	3	Piece 個
magnetic stirring bar 攪拌子	1	Piece 個
magnetic stirrer 攪拌器	1	Set 組
pH meter with electrode 酸鹼度計和電極	1	Set 組
pipette bulb 塑膠吸球	1	Piece 個
Kimwipe papers Kimwipe 拭紙	1	Box 盒
retort stand with clamps 鐵架和夾子	1	Set 組
0.3024 M standardized NaOH solution 0.3024 M 的標準氫氧化鈉溶液	100	ml 毫升
Amino acid Z solution of unknown concentration 未知濃度的胺基酸 Z 溶液	80	ml 毫升

Task I (50 points)

Bacteriophage: an effective agent in the killing of bacteria

噬菌體：一個有效的媒介用於殺死細菌。

Part A. Effects of Phage and antibiotics on the killing of antibiotic-resistant *E. coli* (31 points)

部分 A：噬菌體和抗生素對殺死具抗藥性之大腸桿菌的影響

Introduction

背景知識

A bacteriophage is a virus that infects bacterial cells. Certain bacteriophages can kill bacteria cells by lysis. The bacteriophage is now recognized as an effective agent in the killing of pathogenic bacteria. This provides a good alternative to antibiotics in our combat against disease-causing bacteria that might be resistant to traditional antibiotics.

噬菌體（bacteriophage）是一種可以感染細菌的病毒。某些噬菌體可以透過溶解細菌來殺死細菌。噬菌體目前被認為是一種有效的媒介用於殺死致病的細菌。如此可以作為抗生素之替代方案，來對抗對傳統抗生素具抗藥性之致病細菌。

You are required to design a simple experiment, with proper controls, to examine the killing efficiency of phage of an ampicillin-resistant *E. coli*. Answer the following questions **in the Answer Sheet** and follow the instructions given below.

你被要求設計一個簡單的實驗與適當的控制組，來檢驗噬菌體殺死具ampicillin抗藥性之大腸桿菌的效率。請按照以下的說明與指引，來回答**答案卷**上的問題。

Q1.1 **(1 point)** To dilute the *E. coli* culture from 1×10^7 cells/ml to 2×10^5 cells/ml, what would be the dilution factor needed?

問題 1.1 為了稀釋大腸桿菌溶液從 1×10^7 cells/ml 至 2×10^5 cells/ml，你要稀釋多少倍？（1 分）

Q1.2 **(1 point)** For 1 ml of *E coli* culture at a cell density of 2×10^5 cells/ml, the final concentration of ampicillin used should be 10 μg/ml. What would be the volume of ampicillin stock (1mg/ml) used?

問題 1.2 請問你必須使用多少體積之ampicillin原液 (1 mg/ml)，來配製最終濃度為 10 μg/ml的ampicillin溶液，於 1 ml之大腸桿菌培養液中（最終濃度為 2×10^5 cells/ml）？（1 分）

Q1.3 **(1 point)** For 1 ml of *E coli* culture at a cell density of 2×10^5 cells/ml, final titre of phage used should be. What would be the volume of phage stock (10^8 pfu/ml) used?

問題 1.3 請問你必須使用多少體積之噬菌體原液 (10^8 pfu/ml)，來配製最終效價為 10^6 pfu/ml的噬菌體溶液，於 1 ml的大腸桿菌培養液中（最終濃度為 2×10^5 cells/ml）？（1 分）

Q1.4 **(1 point × 15 = 15 points)** With the above calculated dilution factors, fill in the table **in the Answer Sheet** with your experimental plan. One example is already given for Tube 1 in the table provided. All units are in μl. Carry out your proposed experiment by incubating the four tubes (placed in the labelled floating rack) for 40 minutes (stop watch provided) in the 37 ºC water bath assigned to you. Hand over the floating rack to the technician at the water bath.

After incubation, transfer your samples to the cuvettes labelled 1 to 4. In order to observe the killing of bacteria cells, measure the absorbance at 595 nm wavelength. Bring your samples to the spectrophotometer that is allocated for your use and hand over your samples to the technician. You are to record your own readings as the samples are measured.

問題 1.4 按照上述所計算出之稀釋倍率，將你的實驗設計填入**答案卷**上之表格（15 分）。1 號管（Tube 1）的例子已經幫你填入表格中了。所有填入之數字的單位都以μl表示。執行你的實驗設計來配製這四個管子。並且將這四個管子（放在有標示的圓形微量離心管架），漂浮於 37 ºC的水浴槽中培養 40 分鐘（有提供碼錶）。將插上這四個管子的可漂浮圓形微量離心管架交給水浴槽前的助理人員。

經過 40 分鐘培養之後，將樣品轉移至標示好 1 到 4 的比色管中。為了觀察殺死細菌的效果，請測量 595 nm 的吸光值。帶著你的樣品至你附近的分光光度計，並且交給助理人員。但是！你必須自己記錄每一個樣品的讀值。

Q1.5 **(0.75 × 2 + 1.5 points × 6 = 10.5 points)** Fill in the absorbance reading at 595 nm of the different tubes of reactions in the table provided **in the Answer Sheet**. Taking 1 absorbance unit of the *E. coli* cells at 595 nm to be equivalent to 1×10^7 cells/ml, what are the cell densities of the *E. coli* in the respective reaction tubes?

問題 1.5 填入每一個樣品之 595 nm的吸光值於**答案卷**上。595 nm之吸光值為 1 時的大腸桿菌濃度為 1×10^7 cells/ml。請計算每一個管子之大腸桿菌濃度分別為多少？

Q1.6 **(0.5 points × 5 = 2.5 points)** Which of the following are correct? Indicate correct answer(s) with a tick(✓) and incorrect answer(s) with a cross (×).

a. Due to the ampicillin resistance, the bacteria cell wall prevented easy penetration of the antibiotics, but allowed the phages to enter the *E. coli* cells to cause lysis.

b. The ampicillin resistance in the *E. coli* did not prevent the ability of the phage to adsorp onto the bacteria cells.

c. The bacteriophage likely has a lytic life cycle of around 20 to 30 mins and hence lysis of the *E. coli* was observable during the short experiment.

d. The temperature of 37 ºC was not the correct temperature for ampicillin to kill the *E. coli* cells.

e. The phages competed with the *E. coli* for the nutrients in the LB broth and the bacteria cells lysed due to insufficient nutrients.

問題 1.6 下列問題的敘述正確者請打勾(✓)；錯誤者請打叉(×)。

a. 由於對 ampicillin（抗生素）的抗藥性，細菌細胞壁可以避免抗生素輕易的穿透。

b. 大腸桿菌之 ampicillin 抗藥性無法防止噬菌體吸附在細菌上的能力。

c. 噬菌體似乎約有 20 至 30 分鐘之溶菌生活史，因此可以在短短的實驗中觀察到大腸桿菌的溶解。

d. 37 ºC 不是 ampicillin 抗生素殺死大腸桿菌的正確溫度。

e. 噬菌體與大腸桿菌競爭 LB 培養基內的養分，並且細菌會被溶解是因為養分不夠了所造成的。

Part B. Phage titre and multiplicity of infection (19 points)

部分B：噬菌體效價和感染的多樣性（19分）

The Table below shows the legends for photographs of *E. coli* lawns that are untreated and infected with bacteriophages. The *E. coli* culture used had a starting cell density of 0.5×10^4 cells/ml. 0.5 ml of phage was used to infect the *E. coli* cells. Serial dilutions of the phage culture were made as indicated and used for infection. (The photographs labelled A to H will be provided as part of materials for the lab task).

下表秀出大腸桿菌塗佈培養之照片的圖說：分別為未處理和經噬菌體感染的照片。本實驗所使用之大腸桿菌的起始細胞濃度為 0.5×10^4 cells/ml，並用 0.5 ml的噬菌體來感染大腸桿菌細胞。按照表中之系列稀釋的噬菌體培養液用在此感染實驗。（本實驗任務所提供之照片標示為A至H）

A = 10^{-6} dilution 稀釋	B = 10^{-5} dilution稀釋
C = 10^{-4} dilution稀釋	D = 10^{-3} dilution稀釋
E = 10^{-2} dilution稀釋	F = 10^{-1} dilution稀釋
G = neat phage 僅有噬菌體	H = *E. coli* lawn uninfected by phage 無噬菌體感染之大腸桿菌的塗佈培養

Q1.7 **(2 points × 4 = 8 points)** Based on the number of plaques observed in the photos, calculate the number of plaques that would be observed if the original undiluted phage culture were used.

問題 1.7 根據照片中所觀察到的溶菌斑，請計算當使用未稀釋的噬菌體溶液時，可以觀察到的溶菌斑的數目為何？

Q1.8 **(3 points)** To estimate the titre of a phage culture, serial dilutions as shown in the photos (A to H) are normally performed. Based on the number of plaques shown, indicate with a tick (✓) which is the best dilution to confirm the phage titre.

問題 1.8 為了估計噬菌體培養液的效價，照片 A 至 H 的系列稀釋是被正確進行的。按照溶菌斑的數目，勾選 (✓) 能確認噬菌體效價之最好的稀釋倍率。（3 分）

Q1.9 **(4 points × 2 = 8 points)** Using the information given and your answers above, determine:

a. the plaque forming units per milliliter (pfu/ml) of the phage culture used and
b. the multiplicity of infection (defined as the ratio of phages to *E. coli*) at the best dilution determined in Q1.8..

問題 1.9 使用所提供的資訊和你上述的答案，得出：

a. 所使用之噬菌體為多少 pfu/ml。（4 分）
b. 問題 1.8 所得到之最佳稀釋濃度下之感染多樣性的數值為多少？（感染多樣性的定義為噬菌體濃度除以大腸桿菌濃度的比值）（4 分）

Task II (50 points) 任務二（50 分）

Titration of an Amino Acid

一個胺基酸的滴定

Introduction 背景知識

Amino acids are organic molecules possessing both carboxyl and amino groups. Table 1 shows the 20 amino acids that cells use to build their thousands of proteins.

The majority of the standard amino acids are diprotic molecules since they have two dissociable protons: one on the amino group and the other on the carboxyl group; there is no dissociable proton in the R group.

Recall: For an acid HA, the acid dissociation constant for the equilibrium of $HA \rightleftharpoons H^+ + A^-$ is K_a.

$$K_a = [H^+][A^-] / [HA]$$

More often, the strength of acids is expressed in terms of the pK_a of the acid: $pK_a = -\log K_a$

胺基酸是有機分子，同時擁有羧基（carboxyl groiup）和胺基（amino group）。表 1 秀出 20 種胺基酸，細胞用其來建構成千上萬的蛋白質。

大部分的標準胺基酸為雙氫離子可解離的分子，因為他們有兩個可以解離的氫離子：一個在胺基，另一個在羧基；側基（R group）則沒有可解離的氫離子。

請回想：對一個HA的酸來說，平衡狀況下之HA的解離常數以此表示：$K_a = [H^+][A^-] / [HA]$

酸之pK_a的酸強度以此表示：$pK_a = -\log K_a$

In the titration of such a diprotic amino acid, the titration will thus occur in two steps as the more acidic carboxyl group (lower pK_{a1}) and the less acidic amino group (pK_{a2}) successively lose their protons.

In addition, the pH at which the net charge on the molecule is zero is called the isoelectric point (pI) of the molecule, a useful constant in characterizing and purifying molecules. Using a titration curve,

the pI can be empirically determined as the inflection point between the pK_a's of the anionic and cationic forms.

在滴定一個雙氫離子可解離的胺基酸時，滴定曲線將有兩個步驟：當較酸之羧基 (較小之 pK_{a1}) 和較不酸之胺基 (pK_{a2}) 依序失去他們的氫離子時。

此外，分子淨電荷為零時之溶液pH值即稱為該分子的等電點（pI）。等電點（pI）是一個用於分析和純化分子的有用常數。等電點（pI）可以按照負電性pK_a和正電性pK_a間之滴定曲線的曲折變化，憑經驗來測定其數值。

The apparent pK_a values for the two dissociation steps may be extrapolated from the midpoints of each step. This can be shown by the Henderson-Hasselbach equation:

$$pH = pK_a + \log \{ [A^-] / [HA] \}$$

The pK_{a1} (pK_a for the carboxyl acid group) is where half the acid group has been titrated. Therefore the equation becomes: $pH = pK_a$

Similarly, the pK_{a2} (pK_a for the amino group) can be determined.

In this experiment, you will titrate an unknown amino acid Z and determine its pI, pK_{a1} and pK_{a2}.

兩個解離步驟之明確的pK_a值，可以從每個滴定步驟之滴定中點推得。其方程式（Henderson-Hasselbach equation）以此表示：$pH = pK_a + \log \{ [A^-] / [HA] \}$

pK_{a1}為羧基的pK_a，是酸性基團被滴定的中點。因此方程式可變成：$pH = pK_a$

同樣的，pK_{a2}為胺基的pK_a，也可以按此被測定。

本實驗，你將滴定一個未知的胺基酸Z，並且得出它的等電點pI、它的pK_{a1} 和 pK_{a2}。

Procedure 實驗步驟

1. Fill the burette with the standardized NaOH solution. Record the exact concentration of this standardized NaOH solution **in the Answer Sheet**.

   填充標準氫氧化鈉溶液至滴定管中。紀錄標準氫氧化鈉溶液之濃度至**答案卷**上。

2. Pipette 25 ml of the unknown amino acid solution Z into a clean 100 ml beaker.

   以玻璃吸管吸取 25 ml 的未知胺基酸溶液 Z 至 100 ml 的乾淨燒杯。

3. Carefully place the pH probe and a magnetic stirring bar into the amino acid solution, so that the probe is far enough into the solution, but not touching the stirring bar or beaker. Clamp and adjust the pH probe such that the stirring bar will not hit the probe while stirring. **DO NOT TOUCH THE CALIBRATION.**

   小心地將 pH 電極和一個攪拌子放至胺基酸溶液中。pH 電極必須足夠地伸入溶液中，但是不可碰觸到攪拌子或燒杯。夾住 pH 電極並調整位置，以免攪拌子在旋轉時打到電極。不要碰觸酸鹼度計的校正鍵（CALIBRATION）。

4. Titration 1 滴定 1

   Rinse the pH probe with deionized water. Dry the probe gently with a piece of Kimwipe paper.
   Determine the pH of the amino acid solution Z before the addition of NaOH.
   Next, titrate the amino acid solution with the NaOH from the burette. Add approximately 1.00 ml of the NaOH to the amino acid at a time. Record the exact volume dispensed and the pH of the solution after every 1.00 ml interval **in the Answer Sheet**.
   Continue until approximately 25 ml of NaOH has been added.

   以去離子水浸洗 pH 電極。以一張 Kimwipe 拭紙，輕輕的吸乾 pH 電極。

   加入氫氧化鈉之前，請讀取未知胺基酸溶液 Z 的 pH 值。

   接著以滴定管內的氫氧化鈉滴定胺基酸溶液。每次加入大約 1 ml 的氫氧化鈉至胺基酸溶液中。請在每次加入 1 ml的滴定液之後，紀錄已加入之氫氧化鈉的精確體積，以及溶液的pH值至答案卷上。

   持續滴定與紀錄，直到大約 25 ml 的氫氧化鈉被加至溶液中。

5. Repeat the titration (Titration 2) 重複此滴定實驗（滴定 2）

   Rinse the pH probe with deionized water.

   Dry the probe gently with a piece of Kimwipe paper.

Refill the burette with the standardized NaOH solution and repeat steps 2 – 4.

以去離子水浸洗 pH 電極。

以一張 Kimwipe 拭鏡紙，輕輕的吸乾 pH 電極。

重新將標準氫氧化鈉溶液注入滴定管中，並且重複步驟 2 到步驟 4。

Q2.1 **(3 points × 3 = 9 points)** Table 1 shows the chemical structures of the twenty standard amino acids. With reference to these structures, draw structures to show the complete dissociation of glycine, proline and asparagine.

問題 2.1 表一秀出 20 個標準胺基酸的化學結構。參照這些結構，分別畫出甘胺酸（Glycine）、脯胺酸（Proline）和天門冬醯胺酸（Asparagine）之所有可能的解離結構。（每小題 3 分，共 9 分）

Q2.2 **(3 points × 2 = 6 points)** For both titrations, record the volume of NaOH (ml) added during the titration and the observed pH value for the unknown amino acid.

問題 2.2 進行兩次滴定時，請紀錄加入之氫氧化鈉的體積（ml）和觀察到之未知胺基酸的 pH 值。

Q2.3 **(5 points × 2 = 10 points)** Using your data, plot the graphs of each titration run (pH versus Vol. of NaOH (ml)) in Graphs 1 and 2 provided **in the Answer Sheet**.

問題 2.3 請將你兩次滴定所得到的數據，分別畫於**答案卷**所提供的圖 1 和圖 2（Y軸為pH值，X軸為氫氧化鈉的體積）。

Q2.4 **(2 points × 2 = 4 points)** From your titration curves, find the pI and label it on each graph.

問題 2.4 從你的滴定曲線，找出等電點（pI），並且標示於每一個圖上。(4 分)

Q2.4.1 **(2 points)** What is the mean pI?

問題 2.4.1 你得到的平均 pI 值為何？(2 分)

Q2.5 **(4 points × 2 = 8 points)** Find and label the pK_{a1} and pK_{a2} on each graph.

問題 2.5 找出並標示出每一個圖上的pK_{a1} (4 分) 和 pK_{a2} (4 分)

Q2.5.1 (2 points × 2 = 4 points) What is the mean pK_{a1} and pK_{a2}?

問題 2.5.1 pK_{a1}的平均值為何？ (2 分) pK_{a2}的平均值為何？ (2 分)

Q2.6 **(5 points)** 0.9210 g of the unknown amino acid Z was dissolved in 80 ml of deionized water. Determine the molecular weight of the unknown amino acid Z.

Note: In order to start with a fully protonated amino acid, HCl solution has been added. This is equivalent to 3.2 ml of the NaOH solution. To determine the actual number of moles of NaOH needed to reach the pI, subtract 3.2 ml from the volume of NaOH used to reach the first end point.

0.9210 克的未知胺基酸 Z 被溶解於 80 ml 的去離子水，請計算出未知胺基酸 Z 的分子量。(5 分)

注意：為了讓一開始時之胺基酸是處於完全接上氫的狀況，鹽酸溶液已經被加在樣品當中了。這相當於 3.2 ml 的氫氧化鈉溶液。因此為了決定達到等電點所需之實際氫氧化鈉的莫耳數，請從達到第一個滴定終點的氫氧化鈉體積扣除 3.2 ml。

Q2.7 **(2 points)** Based on Table 2, identify amino acid Z.

依據表二，請鑑定出胺基酸 Z 為何？(2 分)

a. Glycine 甘胺酸

b. Proline 脯胺酸

c. Asparagine 天門冬醯胺酸

d. Tyrosine 酪胺酸

e. Tryptophan 色胺酸

Table 1. Structures of the 20 standard amino acids.

表一：20 個標準胺基酸的化學結構

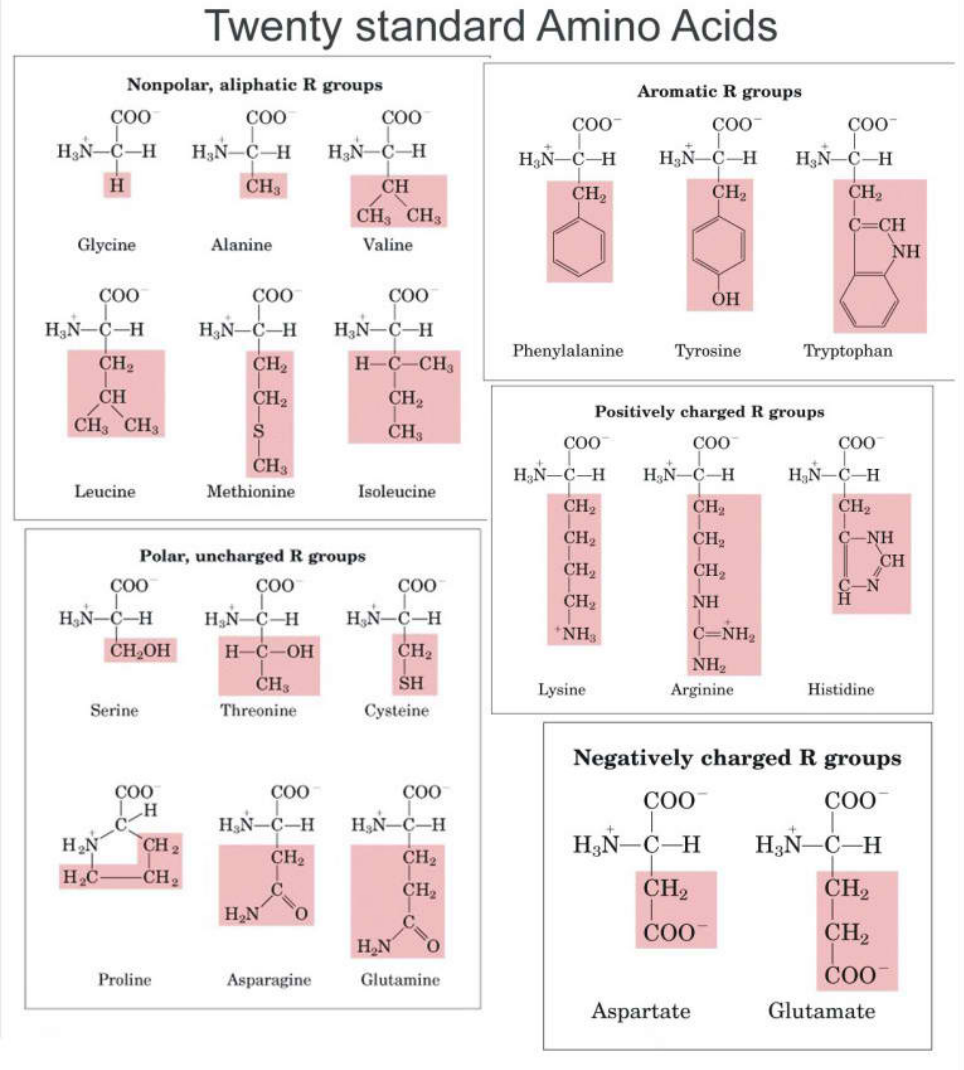

Table 2. Molecular weights of amino acids 胺基酸的分子量

Amino acid 胺基酸	MW (g/mole)分子量
Glycine 甘胺酸	75
Proline 脯胺酸	115
Asparagine 天門冬醯胺酸	132
Tyrosine 酪胺酸	181
Tryptophan 色胺酸	204

END OF PAPER 試題結束