# 30th International Biology Olympiad
# SZEGED, HUNGARY



**Practical Exam 1. 實作一**

**Sugar chromatography 糖類層析法**

**Starch identification 澱粉鑑定**

**Microbial fuel cell 微生物燃料電池**

$16^{th}$ July 2019

| COUNTRY 國家 |
| --- |
| LANGUAGE 語言 |

## Practical exam 1. 實作 1

### General instructions 一般指示

This exam consists of three subtasks: 這個實作測驗包含三個部分：

- Subtask 1. Analysis of sugars with thin layer chromatography (30 points) 使用色層分析來分析糖 (30 分)
- Subtask 2. Identification of a starch of unknown plant origin (30 points) 鑑定不明植物來源的澱粉 (30 分)
- Subtask 3. Microbial fuel cell (40 points) 微生物燃料電池 (40 分)

1. **Please remember to attach your barcode sticker to all pieces of paper on the answer sheet.** 請一定要記得把你的條碼貼紙貼在每一頁答案卷上
2. Write your answers in the separate answer sheet provided. **Only answers given in the answer sheet will be considered**. 把你的答案寫在答案紙上，只有寫在答案紙上的答案才會給分。
3. Ensure you received all necessary materials and equipment listed on the next page. If any items are missing indicate this by raising your red card within 10 minutes following the start of the exam. 請確認你具有下頁所示所有必要的材料與工具。如果有任何缺損請在考試開始後十分鐘內舉起紅卡。
4. During experiments ensure all materials and equipment is handled properly. Any spilled solutions or broken equipment will not be replaced. 在實驗期間請確認所有的材料與工具被適當拿取。任何被弄壞的工具都不會被更新。
5. Stop answering and put down your pencil immediately when the bell rings signalling the end of the exam. 考試結束時請立刻放下筆，停止作答。
6. No paper, materials or equipment should be taken out of the laboratory. 不要把任何紙張、材料與工具帶離實驗室。

CAUTION: The experiment deals with materials that are fragile and sharp. Exercise caution when handling them.

WEAR ALL SAFETY EQUIPMENT PROVIDED AT ALL TIMES OTHERWISE THE LAB ASSISTANT WILL ASK YOU TO LEAVE THE ROOM.

### Materials and Equipment 材料與工具

- eluent solution (boric acid : acetonitrile, 3:7 mixture) in a glass bottle 玻璃瓶中的展開液 (硼酸與乙腈 3:7 的混合液)
- sugar solutions in Eppendorf tubes (G-glucose, M-maltose, S-sucrose, X-honey) 個別包含四種糖類溶液的 eppendorf 管 (G-glucose, M-maltose, S-Sucroce, X-honey)
- Thin Layer Chromatogram (TLC) 薄層色層分析儀
- Pencil 鉛筆
- Pasteur pipettes 巴斯德吸管
- Micropipette tips 微量吸管的 tips
- Screw-cap container 具有旋轉蓋的容器
- Hot air blower (use it at the nearest shared table to detect the developed sugars on the chromatgram) 熱風機 (使用共享桌面上離你最近的熱風機來偵測色層分析後的糖類)
- Gloves 手套
- Ruler 尺

- Lugol solution (in dropper bottle) 魯哥爾溶液 (在滾珠瓶中)
- Microscopy slides 顯微鏡玻片
- Cover slips 蓋玻片
- Knife 刀
- Eppendorf tubes (to be labelled by the student A-D) Eppendorf 小管 (將被標記為 A-D)
- Unknown starch samples (labelled as ” K”) 未知來源的澱粉樣本 (標記為”X”)
- A sachet of dry yeast (Labeled: Y) 一小包乾酵母 (標示為 Y)
- pH=7.0 phosphate buffer (PB) –in a bottle for liquids pH = 7.0 磷酸鹽緩衝液 (PB) 一於裝液體的瓶中
- 0.02 M K3[Fe(CN)6] -solution (Fe3+) dissolved in PB –in a bottle for liquids 0.02 M K3 [Fe(CN)6] 一溶解在 PB 中的溶液（Fe3 +）一於裝液體的瓶中
- Plastic (blue) inoculation loop for mixing the yeast suspension 用於混合酵母懸浮液的塑膠 (藍色) 接種環
- Aqueous citric acid solution for the diaphragm –in a 50 mL-Falcon tube (Labeled “Citric Acid”) 用於隔膜的檸檬酸水溶液一在 50 mL 大離心管中 (標記為“檸檬酸”)
- A strip of filter paper for the diaphragm 隔膜用濾紙條
- 2 x 250 mL plastic cups for assembling the galvanic cell 2 x 250 mL 塑料杯，用於組裝原電池
- Tweezers for soaking and moving the diaphragm 用於浸泡和移動隔膜的鑷子
- Metal clips 金屬夾
- Multimeter with appropriate cables 萬用表配有合適的電線
- Distilled water (W) 蒸餾水 (標記為 W)
- Graphite sheet for electrode (2 pcs) 電極用石墨片（2 個）
- Beaker with room temperature ice 裝有冰塊的燒杯
- Beaker with room temperature water 裝有室溫水的燒杯
- Thermometer 溫度計
- 37 °C dry thermostat (one shared by 4 competitors) 四位選手共用的乾浴槽
- Microcentrifuge tube labelled ‘M’: size ladder marker 標記為 M 的小離心管
- Microcentrifuge tube labelled ‘C’: control which is the PCR product of the original gene 標記為 C 的小離心管 (具有原本基因的 PCR 產物)
- Microcentrifuge tube labelled ‘P1’: PCR product of the sample taken from P1 type cells 標記為 P1 的小離心管 (從 P1 來的 PCR 產物)
- Microcentrifuge tube labelled ‘P2’: PCR product of the sample taken from P2 type cells 標記為 P2 的小離心管 (從 P2 來的 PCR 產物)
- Microcentrifuge tube labelled ‘E’: restriction endonuclease (PstI.) –held on ice! 標記為 E 擺在冰裏面的小離心管 (內有限制酶)
- gel electrophoresis chamber 電泳槽
- electrophoresis gel 電泳膠片

## Part 1. Analysis of sugars with thin layer chromatography (TLC) 使用薄膜色層分析法 (TLC) 分析糖

Different sugars can be readily separated by thin layer chromatography (TLC) analysis. In this task, you have to determine the most abundant sugar component of honey. During the drying and eluting period, you can also start working on the other tasks.

使用薄膜色層分析法 (TLC) 可以分離截然不同的糖，在這實作中，你必須鑑別出蜂蜜中最豐富的糖成分。在乾燥和洗脫過程，你可以開始處理其他實作。

**Be careful with the hot air blower because it can cause severe burns!**
小心熱風機，因為它會導致嚴重灼傷！- 材料清單
**Do not forget you are working with chemicals, always wear gloves!**
別忘了你正使用化學藥品作實驗，一定要戴手套。
**Please remember to attach your barcode sticker to all pages of the answer sheet.**
請記得一定要把你的條碼貼紙貼在所有答案卷上。
MATERIALS and TOOLS 材料與工具:

- eluent solution (boric acid : acetonitrile, 3:7 mixture) in a glass bottle 玻璃瓶中的展開液 (硼酸與乙腈 3:7 的混合液)
- sugar solutions in Eppendorf tubes (G-glucose, M-maltose, S-sucrose, X-honey) 個別包含四種糖類溶液的 eppendorf 管
- Thin Layer Chromatogram (TLC) 薄層色層分析儀
- Pencil 鉛筆
- Pasteur pipettes 巴斯德吸管
- Micropipette tips 微量吸管的 tips
- Screw-cap container 具有旋轉蓋的容器
- Hot air blower (use it at the nearest shared table to detect the developed sugars on the chromatgram) 熱風機 (使用共享桌面上離你最近的熱風機來偵測色層分析後的糖類)
- Gloves 手套
- Ruler 尺

The Eppendorf tubes in the tube rack on your table contain the following samples:

你桌上的微量試管包含以下樣品：

G –glucose 葡萄糖

M - maltose 麥芽糖

S –sucrose 蔗糖

X –honey 蜂蜜

The bottle labelled ’ELUENT’ contains a 3:7 mixture of boric acid solution and acetonitrile.

標註“ELUENT”(展開液) 的罐子含有”硼酸溶液”和”乙腈”的 3：7 混合物。

1. Put on gloves for the exercise!

戴上手套進行操作！

2. Draw a straight line (START LINE) on the white side of the aluminium backed thin layer with a pencil parallel to the shorter side of the sheet, at about 1 cm from its end. Do not push the pencil too hard because otherwise it will scratch off the thin layer!

在鋁背薄膜層的白色面側距離末端平行一公分處畫一直線，請用鉛筆輕觸話線，否則會刮除掉薄膜層！

3. Pipette with a Pasteur pipette a quantity of ELUENT solution in the screw-cap container up to just below the start line, BUT do not place the TLC plate in the it, only verify from the outside that the liquid level is adequate. Screw on the cap of the container and wait 10 minutes for an equilibrium between the eluent and the gas phase above.

使用巴斯德吸管吸取定量的展開液至螺旋蓋容器中開始線正下方為止，請不要將 TLC 板放置其中，而只能從容器外部觀察展開液是否足夠。關上容器蓋後等待 10 分鐘，使展開液與容器氣相之間達到平衡。

4. While waiting, use the Pasteur pipette with a micropipette tip to apply 1 mm of diameter spots of each sugar solution. The spots should be just above the START LINE, with 1 cm far from each other. Use a new pipette tip for each sample.

在等待平衡時，以帶有微量液管尖的巴斯德吸管，使每個糖溶液點塗 1 公釐直徑的圓點。圓點須位於開始線”START LINE”上方，樣本間相距 1 公分，每個樣品皆使用新的微量液管尖。

5. Leave the plate prepared this way on the table.

將準備好的盤子放在桌子上。

6. After visible drying, wait 5 more minutes before proceeding.

在可見的乾燥後，再繼續等待 5 分鐘。

7. Place the TLC plate with the uploaded samples in the container and screw the cap on.

將帶有樣品的 TLC 板放入容器中並擰上蓋子。

8. Continue eluting until the eluent reaches 1 cm distance before the end of the plate. Then remove the plate from the screw-cap container.

持續展開至展開液距 TLC 板頂端約 1 公分距離處。由螺旋蓋容器中取出 TLC 板。

9. Mark the solvent (eluent) front by drawing a line on the TLC plate with a pencil.

用鉛筆在 TLC 板上畫一條線來標註溶劑 (展開液)。

10. Go to the closest free desk with the shared devices. Set the hot air blower to Level II with the and dry the TLC plate by placing it on the tile with its white surface upward and blowing it with hot air.

前往最近的共享桌面使用共享設備，將熱風機設置為 II 級，將 TLC 板以白色薄膜面朝上放在瓷磚上，用熱空氣乾燥。

**Be careful with the hot air blower because it can cause severe burns!**
**小心熱風機，因為它會導致嚴重灼傷！**

**Q.1.1.1** When finished (the TLC has cooled down), put your chromatogram into the envelope labeled “Chromatogram”. **Write the 3-digit number on the envelope in the code box on the Answer Sheet. When you are done, CALL THE LAB ASSISTANT AND SUBMIT YOUR ENVELOPE! (This action is necessary to proceed to the next questions.) 當你完成時 (TLC 已經冷卻)，把你的色層分析圖擺進標記為”Chromatogram” 的信封，把答案卷上的三位數代碼寫在信封上。當你完成時，請通知實驗助教並繳交信封 (如果沒有完成這個動作就無法回答下一個問題)。**

**When you submit your own chromatogram image, the lab assistant will give you a new one required to complete the next tasks. (This chromatogram was run in the same manner as the one performed earlier.**

**當你完成要繳交色層分析圖的影像時，實驗助教會給你一個可以完成下一個實作所需的新層析圖像 (這個色層分析圖是使用上一個一樣的流程做出來的)。**

Based on the provided chromatogram image, perform the following measurements and then calculate the retention factors (Rf). Use the widest part of each spot for measuring the distance.

依據獲得的層析圖，執行以下測量，然後計算滯留因子 (Rf)。使用每個斑點的最寬部分來測量距離。

The distance of solvent front (‘ELUENT’) from the ‘START’ line: _____ cm

溶劑前沿 (’ 展開液’) 距離’ 開始線’ 的距離：_____ cm

$$R_f = \frac{d}{E}$$

Where:

d: distance of the spot from the START line 斑點距離開始線的距離

E: distance of ELUENT front from the START line 展開液前端距離開始線的距離

**Q.1.1.2** Fill the TABLE 1 on the answer sheet.
填寫答案紙上的表 1。

**Q.1.1.3** Based on the chromatogram given, identify the carbohydrate(s) present in the honey sample. Put an X under the labels (G, S, M) of the appropriate sugars.
根據所獲得的層析圖，鑑定蜂蜜樣品中所存在的碳水化合物。在適當的糖之標籤 (G、S、M) 下面標註一個”X”

## SUBTASK 2. IDENTIFICATION OF A STARCH OF AN UNKNOWN PLANT ORIGIN 鑑定來自不明植物的澱粉

In this practical your task will be to study and draw several types of starch grains.

在本題中，你將研究和畫出幾種類型的澱粉粒。

1. Using your knife scrap the surface of your biological samples onto separated microscopic slide each. (A: bean, B: wheat grain, C: potato, D: rice grain) Write the appropriate letter (A, B, C or D) on the slide with the marker.

用你的小刀刮取下列材料的表面並塗抹到不同的顯微鏡載玻片上。(A：豆子，B：小麥粒，C：馬鈴薯，D：米粒）並用麥克筆在載玻片上寫下相對應的字母（A，B，C 或 D）。

2. Drip one drop of Lugol’s solution (iodine solution with KI) on the scrapings, cover it with a cover slip and examine them at a 100x total magnification under a microscope.

在刮取的組織上滴一滴魯哥爾溶液（含 KI 的碘溶液），蓋上蓋玻片，在顯微鏡下以 100 倍之放大倍率檢查。

**Q.1.2.1** (No marks allocated for this point, nevertheless if your sample is not approved it means that you can’t get more marks in this task.)
(本部份無配分，但如果你的樣本不合格，你也無法在此題中得分。)
When all the slides are prepared, adjust a scraping of your choice to sharp resolution and call the lab supervisors to prove your work.
製備好所有組織切片後置於顯微鏡下觀察，得到清晰的影像後，告知實驗監督人員以便檢測你的結果是否合格。
Be aware that you need these slides for the next tasks. 請注意，你需要這些組織切片以完成下一題。

**Q.1.2.2** Draw at least two starch grains per each sample (A-D). Make sure you draw each starch grain in sufficient detail, they should reflect the shape, pattern, and (relative) size of each grain. 至少從樣本 A 到 D 中畫出兩種在顯微鏡下所觀察到的澱粉粒。確定你畫得夠仔細，結果圖應反映不同樣品的顆粒的形狀、紋路、還有相對尺寸。

You have received a food starch sample, used in the food industry as a thickener, in an Eppendorf tube (labelled as ‘K’). Test a very small portion of this, by diluting with drops of water and Lugol’s solution.

你已經接收到食品工業中常用來當作食品增稠劑的澱粉樣品 (置於微量離心管中，標記為“K”)。可利用水和魯哥爾溶液之稀釋來測試此樣品其中的一小部分。

**Q.1.2.3** Based on your observations from the previous task, determine from which plant the sample came. Indicate your answer by putting an X in the appropriate box(es) on your answer sheet. Note that putting an X into any inappropriate box causes loss of half of the total marks for Q.1.2.3. until you reach zero. 根據你的發現，請指出那個樣本是從什麼植物來的。在適當的答案格畫 X。答錯會導致 Q.1.2.3 的總分數損失一半，直到零分。

**Q.1.2.4** Assume that the average volume of a starch grain is $4000\ (\mu m)^3$ . The starch grain can be considered a 20% aqueous solution of starch with a density of 1.050 g/$cm^3$. What is the weight of the starch constituting this one grain? Give you answer in mm rounded to the nearest integer ($1\ \mu m\ = 10^{-6}\, m$).
假設澱粉粒的平均體積是 4000 µm3 。澱粉粒可視為是 20%, 密度為 1.050 g/cm3 的澱粉水溶液。構成這一粒澱粉的重量是多少？以 mm 為單位，並以最接近的整數寫出你的答案。

**Q.1.2.5** How many glucose subunits does the above amount of starch grain consist of? Assume that the grain consists of amylose only, with an average number of 1000 subunits per molecule. Give the result with 3 significant figures in scientific notation. The molar mass of glucose is 180 g/mol; that of water is 18 g/mol; Avogadro number (NA): $6 \cdot 10^{23}$ 1 /mol. 上面的澱粉含量是多少分子？假定這些顆粒只包含了直鏈澱粉，一個分子平均有 1000 個次級單位。用科學計數法給出 3 個有效數字的結果。葡萄糖的分子量為 180 Da; 水的分子量是 18 Da; 亞佛加厥數（NA）: $6 \cdot 10^{23}$ 1 /mol.

## SUBTASK 3. MICROBIAL FUEL CELL 微生物燃料電池

**DURING THIS PRACTICAL YOU WILL WORK WITH CARBON TISSUE. BE AWARE THAT IT IS EXTREMELY DANGEROUS TO HANDLE IT WITHOUT GLOVES!**
在此實作，你將使用碳組織。請注意，在沒有手套的情況下操作是極端危險的！

Methylene blue is a widespread dye, also used as a redox indicator in addition to dyeing microscopic preparations and macromolecules. Since this material is not toxic at a low concentration, bacterial and yeast cells can absorb it without suffering significant damage. However, due to its redox properties and good diffusion abilities, it is known that at an appropriate concentration is able to intervene in redox processes in cells - "tapping" electrons from the latter. For example, its ability to gain electrons from glucose, thus converted to gluconic acid, is well known. In this task we try to discover whether it is capable of achieving this with a material participating in the oxidative phosphorylation (terminal oxidation) process in the mitochondria. In fact, methylene blue does not enter the mitochondria, but if its potential effect could be proven, a small modification of the molecule could achieve an effective short-circuit in oxidative phosphorylation, thus it could serve as a potential starting point for drug development as well. Hungarian researchers published a paper in 2017 (Komlódi, Tretter; 2017), outlining a possible model of a mechanism in which methylene blue can help neurons get ATP in some neurodegenerative diseases (e. g. Alzheimer's disease).

亞甲藍是廣泛應用的染劑，除了用來做為顯微切片備製及巨大分子的染色之外，也可當作氧化還原反應的指示劑。由於此材料在低濃度時不具毒性，細菌和酵母菌細胞能吸收亞甲藍而不會遭到明顯損害。然而，由於其氧化還原特性以及良好的擴散能力，在適當濃度下，亞甲藍能干擾細胞中的氧化還原過程－從細胞中"敲掉"電子。例如，亞甲藍從葡萄糖獲得電子的能力並使之轉為葡萄糖酸的過程，已被清楚了解。在此實作中，我們將藉由在在粒線體中，亞甲藍在氧化磷酸化 (末端氧化作用) 的參與，嘗試探討它是否能完成上述過程。事實上，亞甲藍並不進入粒線體，但是倘若其潛在作用獲得證實，分子的小幅變型能達成在氧化磷酸化有效的捷徑，因此它也能在藥物開發時，當作潛在的起始點。匈牙利研究人員在 2017 年發表一篇論文 (Komlódi, Tretter; 2017)，概略地提出一個有關亞甲藍能幫助神經元在一些神經退化疾病 (如阿茲海默症) 中得到 ATP 的可能模式。

Your task is now to test the electrode potential (mid-point potential) of methylene blue, thus confirming or rebutting that methylene blue is indeed capable to participate in the above process in this way. You will make your decision on the basis of the following chemical principle: an agent with the more negative redox potential is able to reduce (transfer an electron to) the agent with the more positive redox potential.

現在，你的任務是測試亞甲藍的電極電位 (中點電位)，以確定或反駁亞甲藍的確參與如前所述的過程。你將根據下列的化學原理來做決定：一個具有更多負氧化還原電位值者，能還原 (轉移電子) 具有更多正氧化還原電位值者。

The mid-point potential is the electrode potential value measured when a reduced and oxidized form of a redox agent is present in equal amounts. In the reaction you perform, this type of equilibrium is reached by a galvanic cell and yeast cells. Living cells continuously reduce methylene blue, the reduced form of methylene blue is released from the cells, where they will form the anode of the galvanic cells, continuously supply the appropriate electrode with electrons. Oxidation, electron transfer of methylene blue is also continuous on the electrode, thus the ratio of the reduced and oxidized forms is constant in both the cells and the fluid surrounding the electrode, the mid-point potential can be determined as the solution's electrode potential. 中點電位是當一個氧化還原劑以等量的還原與氧化型式存在時，所測得的電極電位值。在你將操作的反應中，這樣的平衡可由伽伐尼電池 (galvanic cell ) 和酵母菌細胞，活細胞持續還原亞甲藍，此亞甲藍的還原態可從細胞中釋出，其將會形成伽伐尼電池的陽極，持續提供具有電子的適當電極，因此在兩種細胞以及電極周圍的液體中的還原和氧化態比值是恆定的，中點電位可被定為溶液的電極電位。

Even at this time, the galvanic cell will consist of two electrodes: methylene blue release an electron on one electrode (anode) and K3[Fe(CN)6] (Fe3+) takes up an electron on the other one (cathode) and is thus converted to K4[Fe(CN)6] containing ferrous (Fe2+) ions . The galvanic cell is kept “alive” while oxidized methylene blue molecules are able to enter the yeast cells where they are reduced in the above-mentioned redox reaction, so the galvanic cell is not depleted as long as there are live yeast cells and enough Fe3+ ions. 即使在此次，伽伐尼電池將包括兩個電極：亞甲藍在一電極 (陽極) 釋出電子，而在另一電極 K3[Fe(CN)6] (Fe3+) 則吸收一個電子 (陰極)。當氧化的伽伐尼電池被維持”活著”，故而轉化成 K4[Fe(CN)6]，內含鐵離子 (Fe2+)。伽伐尼電池被維持” 活著”，而氧化亞甲藍分子能進入酵母細胞，並如上述的氧化還原反應中，在細胞內還原，故只要還有活的酵母菌和足夠的鐵離子 (Fe3+)，伽伐尼電池不會耗盡。

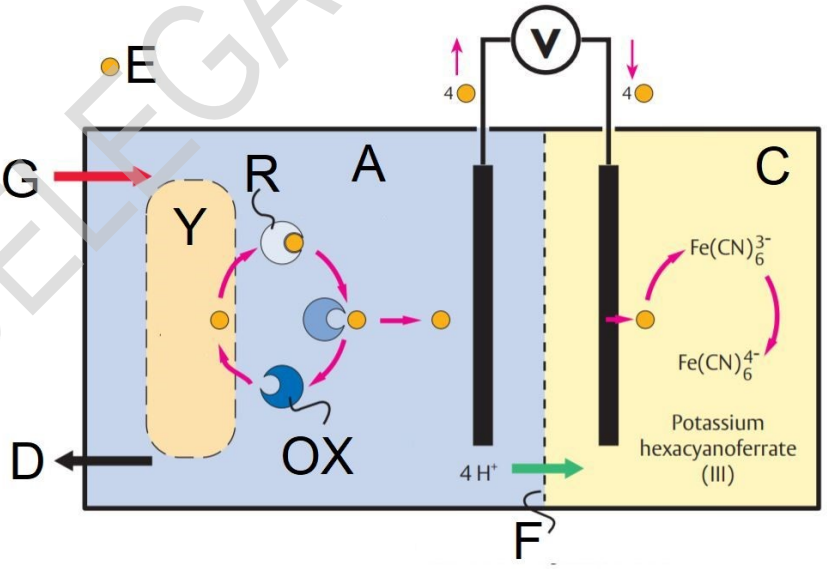


Fig.1. Legend:A: anode. C: Cathode. D: CO2. E: electron. F: Diaphragm connecting the two electrolites. G: Glucose. OX: oxidized form of methylene blue. R: Reduced form of methylene blue. Y: Yeast cell.
圖 1。圖說：A：陽極。C：陰極。D：CO2。E：電子。F：連接兩個電解質的隔膜。G：葡萄糖。OX：氧化形式的亞甲藍。R：亞甲藍的形式減少。Y：酵母細胞。

Your task is to assemble this live galvanic cell and to measure its electromotive force. After you showed

the results of your measurement, you will receive information on a separated sheet to be used at the calculation tasks. 你的任務是將此活的伽伐尼電池組合起來，並測量其電動勢。當你呈現測量結果之後，你將收到另一張表格紙上的資訊以用來進行計算。

**Assembly of a galvanic cell:** 組裝一個伽伐尼電池：

**To measure volumes use the scaling on the wall of the cups (in mL-s).** 使用杯子壁上的刻度（以 mL-s 為單位）來測量體積。
**You should use the two scaled colourless plastic cups in the following procedure. Put Both into the white rectangular box before you start to create the fluid of the anode and the cathode.** 您應該按照以下步驟使用兩個無色塑料杯。在開始製作陽極和陰極的液體之前，將兩者放入白色方形盒中。

**When preparing the anode fluid, follow the steps below:** 準備陽極液時，請按照以下步驟操作：

1. Pour about 20 mL of phosphate buffer (PB) to a 250 mL cup and label it as ‘ANODE’ 將約 20 mL 磷酸鹽緩衝液 (PB) 倒入 250 mL 杯中，並將其標記為“ANODE”
2. Add the total amount of the provided powdered yeast to the buffer. 將提供的粉末酵母的總量添加到緩衝液中。
3. Stir the mixture with the inoculation loop until a suspension with uniform texture (homogeneous) is formed. 用接種環攪拌混合物直至形成具有均質的懸浮液。
4. Add about 20 mL of glucose solution (G) to the mixture obtained thereby further homogenizing the yeast suspension. 在所得到的混合物中，加入約 20 mL 葡萄糖溶液 (G)，並將酵母懸浮液均質化。
5. Then add 20 mL of methylene blue solution (M) to the mixture thereby further homogenizing the suspension. 然後向混合物中加入 20 mL 亞甲基藍溶液 (M)，並將酵母懸浮液均質化。

**When preparing the cathode fluid, follow the steps below:** 製備陰極液時，請按照以下步驟操作：

1. Pour 60 mL of yellow K3[Fe(CN)6] solution (Fe3+) into the other glass vessel. 將 60 mL 黃色 K3 [Fe(CN)6] 溶液 (Fe3 +) 倒入另一個玻璃容器中。

**When assembling the diaphragm, follow the steps below:** 組裝隔膜時，請按照以下步驟操作：

The diaphragm is intended to provide electrical connection between the two solutions (i. e. it has to reach down into both solutions.) This function will be fulfilled by a filter paper strip soaked in citric acid solution in this case by means of highly mobile hydrogen ions in the solution. 示意圖可提供兩種溶液間的電流連接 (即：它必須連到兩種溶液）。此功能將透由浸泡在檸檬酸溶液中的濾紙條來完成，在此處，即是藉由溶液中可高度移動的氫離子來進行。

1. Put the cathode fluid on the right and the anode fluid on the left. 將陰極液放在右側，將陽極液放在左側。
2. Put the two cups of your forming galvanic cell next to each other so that their edges come into contact. 在形成伽伐尼電池中，將兩個杯子放一起，使它們的邊緣接觸。
3. Fold the filter paper in half along its shorter side (i. e. making it half as long as before). 將濾紙沿其較短的一側折疊成兩半（即：使其長度為原先的一半）。
4. Then, completely dip the prepared filter paper strip into the citric acid solution by using the tweezers and let it soak for ca. 5 seconds. 然後，用鑷子將製備好的濾紙條完全浸入檸檬酸溶液中，然後浸泡約 5 秒鐘。
5. Lift out the moistened filter paper strip, let it drain off, then place it in the two plastic cups, so that the two tips hanging down into each of the solutions. 取出濕潤的濾紙條，瀝乾，然後將其放入兩個塑膠杯中，使濾紙條的兩個尖端垂下來，並分別浸入兩種溶液中。
6. This is important, because the filter paper should not come into contact with the graphite electrodes as this would cause a short circuit. 這很重要，因為濾紙不應與石墨電極直接接觸，否則會導致短路。

**Assembly of a galvanic cell –measurement of the electromotive force:**

伽伐尼電池組裝－電動勢的測量：

1. Now, only the electrodes should be placed in both solutions prepared this way, then connected to the voltage meter. To arrange the voltaic cell you can use all the tools provided. 現在，只有電極應放在所製備的兩種溶液中，然後連接到電壓表。要組成伏打電池 (voltatic cell)，您可以使用提供的所有工具。
2. IF YOU HAVE FINISHED UP TO THIS POINT, RAISE YOUR **YELLOW CARD**, AND HAVE YOUR ASSEMBLED SYSTEM CHECKED BY YOUR LAB ASSISTANT, WHO WILL SIGN YOUR ANSWER SHEET. 如果您已完成組裝至此，請舉起黃卡，請實驗室助理檢查您的組裝系統，他/她會在您的答案紙上簽名。
3. Take off the caps from both the black and the red cables of the multimeter. 從萬用表的黑色和紅色電線上取下蓋子。
4. Take the multimeter’s black cable into your left hand and the mutimeter itself into your right hand. 將萬用表的黑色電線放在左手，將萬用表放在右手。
5. Switch on the multimeter with turning the black wheel on it to the ‘V’ sign. 打開萬用表，將黑色滾輪轉到“V”標誌。
6. Touch the two carbon electrode with the two needle points and read the voltage. 用兩個針尖觸摸兩個碳電極並讀取電壓。

**Q.1.3.2** Record the measured voltage on the Answer Sheet. 在答題紙上記錄所測量的電壓。

After you recorded your results a scotch tape will be stacked on your Answer Sheet. You also receive a Voltage value with which you have to calculate in the following tasks. 記錄結果後，將在您的答題紙上堆放透明膠帶。您還會得到一個電壓值，您必須據此來計算以下的題目。

**Calculation tasks for the microbial fuel cell 微生物燃料電池的計算**

You received a voltage value from the lab assistant you have to calculate with! 從實驗室助教得到的電壓值，據以進行計算！

The following are known:

下面為已知條件：

- The standard redox potential of the [Fe(CN)6]3- +e- = K3[Fe(CN)6]4- reaction: 0.361 V
- Molar mass of K3[Fe(CN)6] : 329.24 g/mol
- Molar mass of methylene blue: 319.85 g/mol
- The K3[Fe(CN)6] -solution was prepared by dissolving 3.39 g of salt in 500 mL of PB.
- The methylene blue solution was prepared by dissolving 1.87 g of dye in 500 mL of PB.
- Calculation of electrode potentials of solutions with an approximate concentration of 1 $\frac{mol}{dm^3}$ is specified by the Nernst equation:

Fe(CN)6 3- + e- = K3 [Fe(CN)6] 4-反應的標準氧化還原電位：0.361 V。K3 [Fe(CN)6] 的摩爾質量：329.24 g / mol

- 亞甲基藍的摩爾質量：319.85 g / mol
- 將 3.39 g 鹽溶解在 500 mL PB 中來製備 K3 [Fe(CN)6] 溶液。
- 將 1.87 g 染料溶解在 500 mL PB 中來製備亞甲基藍溶液。
- 由能斯特方程式來計算濃度約為 1 的溶液的電極電位：

$$\varepsilon = \varepsilon_0 + \frac{RT}{zF} \ln c$$

Where:

- ε: the cell potential
- ε0: the standard redox potential
- R: the universal gas constant (8.314 J/mol K)
- T: the absolute temperature
- F: the molar charge (96486 C/mol)
- z: the number of moving electrons (methylene blue also loses/gains four electrons per round)
- c: concentration expressed in mol/L
- ε：細胞電位
- ε0：標準的氧化還原電位
- R：通用氣體常數（8.314 J / mol K）
- T：絕對溫度
- F：摩爾電荷（96486 C / mol）
- z：移動電子的數量（在每次迴路，亞甲藍也會 失去/獲得 四個電子）
- c：以 mol / L 表示的濃度

A measurement performed under optimal circumstances was completed with the following results: 完成在最佳情況下進行的測量，結果如下：

- The temperature was 25 °C 溫度為 25°C
- 10% of the methylene blue was present in the anode solution –the rest was in the yeast cells. 陽極溶液中存在 10%的亞甲基藍 - 其餘的存在於酵母細胞中。
- Electromotive Force (EMF) is the voltage you have measured. 電動勢（EMF）是您測量的電壓。

**Q.1.3.3** What is the value of the RT/F multiplication factor for both electrodes (calculated in SI units)? Give your answer to 5 decimal places on the answer sheet.
兩個電極的 RT / F 倍增係數的值（以 SI 單位計算）是多少？在答題紙上給出 5 位小數的答案。

**Q.1.3.4** What is the electrode potential of the cathode solution in this setup? Give your answer in millivolts rounded to an integer.
在這種設置中，陰極溶液的電極電位是多少？以毫伏為單位，四捨五入至整數。

**Q.1.3.5** What is the electrode potential of the anode solution in this setup? Give your answer in millivolts as an integer.
在這種設置中，陽極溶液的電極電位是多少？以毫伏為單位，四捨五入至整數。

**Q.1.3.6** Enter the concentration of methylene blue in the anode solution. Give your answer in mol/L rounded to 2 significant figures.
寫出陽極溶液中亞甲基藍的濃度。以 mol / L 給出你的答案，四捨五入到 2 位有效數字。

**Q.1.3.7** Enter the standard redox potential of methylene blue based on the measurement data. Give your answer in millivolts as an integer. 根據測量數據輸入亞甲基藍的標準氧化還原電位。答案以毫伏為單位。

Based on another calculation, the midpoint potential of methylene blue was 11 mV. The table below shows the standard redox potentials of biological oxidation processes. Based on the midpoint potential value of methylene blue and the figures in the table below, write X in the appropriate cell(s) on the answer sheet.

根據另一個計算，亞甲藍的中點電位為 11 mV。下表顯示了生物氧化過程的標準氧化還原電位。根據亞甲基藍的中點電位值和下表中的數字，在答案紙上的相應單元格中寫入 X.

Methylene Blue is capable of …

亞甲藍能夠.....

A. taking up electrons from

從... 帶走電子

B. transferring electrons to

轉移電子到...

**Q.1.3.8** NADH

**Q.1.3.9** Cytochrome C
細胞色素 C

**Q.1.3.10** Succinate
琥珀酸

| Electrode equation | $E'_0$(V) |
|---|---|
| $Succinate + CO_2 + 2\,H^+ + 2\,e^- \rightleftharpoons \alpha\text{-ketoglutarate} + H_2O$ | −0.670 |
| $Acetate + 2\,H^+ + 2\,e^- \rightleftharpoons acetaldehyde$ | −0.580 |
| $2\,H^+ + 2\,e^- \rightleftharpoons H_2$ | −0.421 |
| $\alpha\text{-Ketoglutarate} + CO_2 + 2\,H^+ + 2\,e^- \rightleftharpoons isocitrate$ | −0.380 |
| $Cystine + 2\,H^+ + 2\,e^- \rightleftharpoons 2\,cysteine$ | −0.340 |
| $NAD^+ + 2\,H^+ + 2\,e^- \rightleftharpoons NADH + H^+$ | −0.320 |
| $NADP^+ + 2\,H^+ + 2\,e^- \rightleftharpoons NADPH + H^+$ | −0.324 |
| $Acetaldehyde + 2\,H^+ + 2\,e^- \rightleftharpoons ethanol$ | −0.197 |
| $Pyruvate + 2\,H^+ + 2\,e^- \rightleftharpoons lactate$ | −0.185 |
| $Oxaloacetate + 2\,H^+ + 2\,e^- \rightleftharpoons malate$ | −0.166 |
| $FAD + 2\,H^+ + 2\,e^- \rightleftharpoons FADH_2$ (in flavoproteins) | +0.031 |
| $Fumarate + 2\,H^+ + 2\,e^- \rightleftharpoons succinate$ | +0.031 |
| $Ubiquinone + 2\,H^+ + 2\,e^- \rightleftharpoons ubiquinol$ | +0.045 |
| $2\ cytochrome\ b_{(ox)} + 2\,e^- \rightleftharpoons 2\ cytochrome\ b_{(red)}$ | +0.070 |
| $2\ cytochrome\ c_{(ox)} + 2\,e^- \rightleftharpoons 2\ cytochrome\ c_{(red)}$ | +0.254 |
| $2\ cytochrome\ a_{3(ox)} + 2\,e^- \rightleftharpoons 2\ cytochrome\ a_{3(red)}$ | +0.385 |
| $\frac{1}{2}O_2 + 2\,H^+ + 2\,e^- \rightleftharpoons H_2O$ | +0.816 |

Table 1. Standard Redox Potentials of Selected Half-Reactions

表 1. 挑選半反應的標準氧化還原電位