Country Code: _____ Country: _____

20th INTERNATIONAL BIOLOGY OLYMPIAD
12th – 19th July, 2009
Tsukuba, JAPAN

Directions :

- You should not open the envelope until the bell rings once to indicate the start of the test.
- After the bell rings, please open the envelope and write your student code on every page of the ANSWER SHEET at the beginning of the test.
- 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.
- When the bell rings twice to indicate the end of the test, please put down your pencil and stop writing.
- For safety reasons, do not take any food or drink into the laboratory.
- You must wear your coloured laboratory coat together with appropriate clothes and shoes.

・Please properly use the materials (pencils, a pencil sharpener, an eraser, a ruler, a marker pen, a stopwatch, goggles, gloves, a calculator) which were given to you at registration.

・Distilled water (DW) in a bottle, paper towels, cleaning papers and two plastic cups for discarding liquid and solid materials have been provided at your bench. Please use them as needed.

・After the test, please be sure that you have cleaned the bench before you leave.

How to handle a micropipette:

Each micropipette has a fixed range of volumes as indicated on the head of pipette. Please use appropriate types of the micropipettes. Do not cross the limits of this range.

Type (Volume)	Head	Window	Volume
P1000 (200-1000 microlitre)			indicates 850 microlitre
P200 (50-200 microlitre)			indicates 150 microlitre
P20 (2-20 microlitre)			indicates 15 microlitre
P2 (0.2-2 microlitre)			indicates 1 microlitre

Volume adjustment: turn the dial (1) to set the value to the desired volume, which can be seen in the window.

Use: Secure the pipette tip to the tip holder (2). Gently push down the plunger (3) to the first stop, hold, and dip the tip into the solution vertically to a depth of 2 - 4 mm. Release the plunger slowly and make it return to the original position. Remove the pipette from the liquid and transfer the contents to the desired tube. Make sure that the tip is close to the inner wall of the tube. Push the plunger to the first stop and then push further to discharge the solution completely from the tip. Remove the pipette from the tube. Put the used tip into the disposal container by pressing the tip-ejector (4).

Attention: With the 200-1000 microlitre pipette (P1000), it may a suck the solution into the pipette cylinder with the rapid release of the plunger. If this happens, please tell the help staff after the test.

Student Code: _____

20th INTERNATIONAL BIOLOGY OLYMPIAD

12th – 19th July, 2009

Tsukuba, JAPAN

PRACTICAL TEST 2

BIOCHEMISTRY

Total Points: 100

Duration: 90 minutes

Dear Participants,

- In this test, you have been given the following 2 tasks:

  Task 1: Measurement of acid phosphatase activity (70 points)

  Task 2: Protein determination (30 points)

- **You must write down your results and answers in the ANSWER SHEET. Answers written in the Question Paper will not be evaluated.**
- 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.
- At the end of the test, put the Answer Sheet and Question Paper in the envelope. The supervisor will collect this envelope.

Good Luck!!

How to use the spectrophotometer

1. The screen of spectrophotometer (Shimadzu UVmini-1240) must show 400 nm (Fig. 1). If not, raise your hand. ABS value shown may not be 0.000.
2. Fill a plastic semi-micro cuvette with distilled water (DW) at least up to the shoulders inside (Fig. 2)
3. Insert the cuvette into the cuvette holder of the instrument, with the transparent surfaces facing to the left and right (Fig. 3).
4. Shut the lid (Fig. 4).
5. Press ‘AUTO ZERO’ button (Fig. 5). By this manipulation, the instrument regards the level of absorbance by the cuvette plus water as zero. This will be used as the blank control for the rest of this experiment.
6. Now, you are ready to measure absorbance of samples.
7. Replace the water with a sample solution and read an ABS value after the lid is shut. The absorbance is caused by solutes in the sample solution.
8. You do not have to wash the cuvette after every measurement, if you measure a series of samples from the dilute to the concentrated.

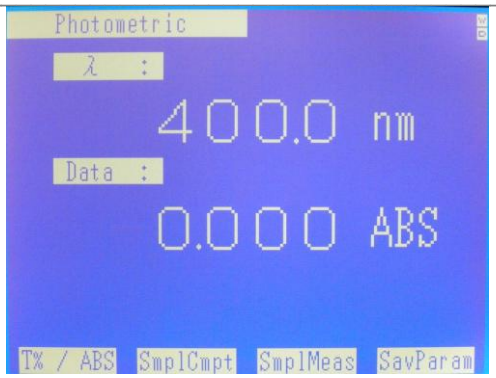


Fig. 1

Fig. 2

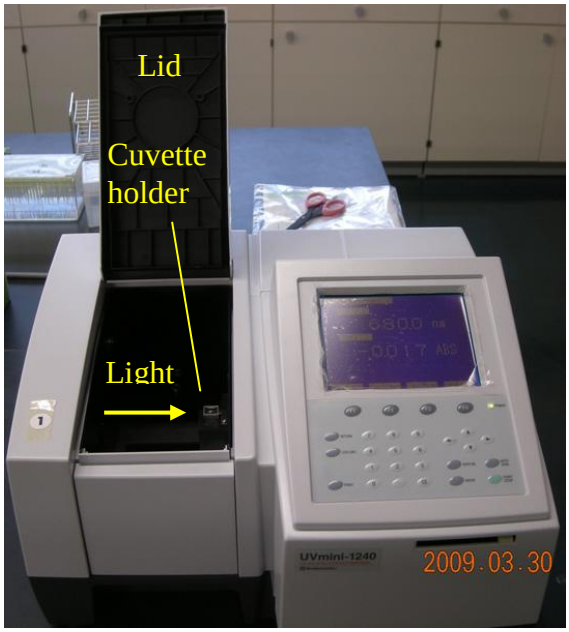


Fig. 3

Fig. 4

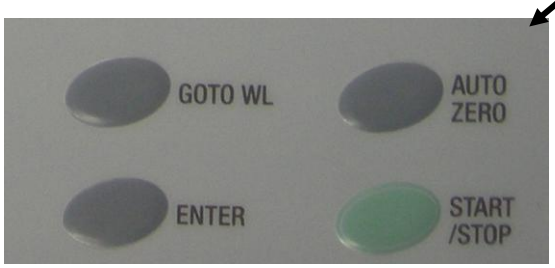


Fig. 5

Introduction

Acid phosphatase liberates phosphate ions from phosphorylated molecules under acidic conditions. The purpose of this experiment is to determine the specific activity of the acid phosphatase. You will measure activities of the acid phosphatase using a crude extract from potato in Task 1, and determine a protein concentration of the crude extract in Task 2. Specific activity, which is the activity per unit time per unit weight of protein, is obtained from Tasks 1 and 2. Specific activity is an index of purity; it increases as the enzyme is purified.

Caution

1. You will be handling small amounts of toxic substances (*p*-nitrophenol and NaOH).You can choose to wear disposable gloves and safety goggles in the experiments if you like.
2. In calculations where answers to previous questions are needed, partials marks will be given if calculated formulas are correct, even if answers are incorrect.

Materials and Equipments	Quantity
1. Spectrophotometer	1
2. Micropipettes (P1000)	2
3. Micropipettes (P200)	1
4. Tips (one box each for P1000 and P200)	2
5. Plastic cuvette	1
6. Test tube holder that accommodates 6-1 to 6-6	1

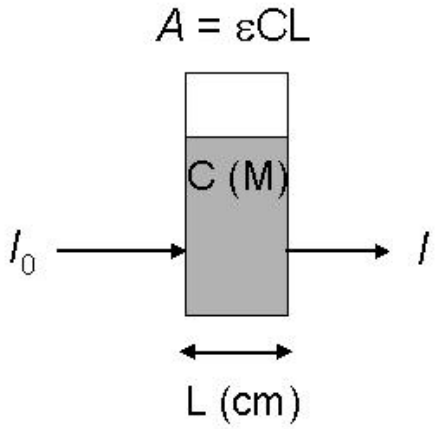
6-1. Crude extract of acid phosphatase (4 ml in a 15-ml plastic tube, labeled '1x enzyme')	1
6-2. 0.5 M Na acetate buffer (pH 5.6) (2 ml in a 15-ml plastic tube)	1
6-3. 5 mM pNPP (4 ml in a 15-ml plastic tube)	1
6-4. 0.5 M NaOH (8 ml in a 15-ml plastic tube)	1
6-5. 3% NaCl (10 ml in a 15-ml plastic tube)	1
6-6. Test tubes (Glass)	6

Task 1 (70 points)

Measurement of acid phosphatase activity

The activity of acid phosphatase is measured by an enzymatic reaction that converts *p*-nitrophenyl phosphate (pNPP) to *p*-nitrophenol (pNP), liberating phosphate. The product, pNP, absorbs light whose wavelength is 400 nm with an absorption coefficient*($\varepsilon_{400\ nm}$) of 19000 M^{-1} cm^{-1} at extremely alkaline pH. Reaction mixture for an acid phosphatase is slightly acidic. Thus, it must be alkalinized for quantification of pNP. In Task 1, you will measure a time course of the reaction and obtain absorbance change per minute that is caused by 1 ml of crude extract. The absorbance change is converted to concentration change by using $\varepsilon_{400\ nm}$. Then, you will calculate a mol number of pNP molecules produced during the reaction by multiplying the concentration change by a volume of sample that is subjected to the measurement of absorbance.

*What is an absorption coefficient ?



A, absorbance

ε, absorption coefficient (M^{-1} cm^{-1})

C, concentration (M=mol $litre^{-1}$)

L, light path length (cm)

I_0, intensity of incident light

I, intensity of transmission light

Absorbance (*A*) is a physico-chemical property of solution that expresses to what extent a solute absorbs light at a specific wavelength. Absorbance is in proportion to concentration (C) and light path length (L). The constant in the equation is a value characteristic to the solute, and is termed the absorption coefficient (ε). Thus, the relationship is formulated as $A=\varepsilon$ C (M=mol $litre^{-1}$) L (cm). Absorbance can be converted to concentration, since ε is given and L is 1 cm in this experiment. The dimension of ε is M^{-1} cm^{-1}, because absorbance is an absolute number without units.

Two enzyme concentrations are to be examined in Task 1. Find the test tube on which ‘1x enzyme’ is labeled, which contains a crude extract of acid phosphatase. Next, find the 15-ml tube that contains 3% NaCl and remove 1ml of the solution so that the tube now contains 9 ml of 3% NaCl. Add 1 ml of the ‘1x enzyme’ solution to it by using a micropipette, which makes ‘0.1x enzyme’ solution. Relabel the tube as ‘0.1x’. Next, find 6 empty test tubes. Label each tube with an enzyme concentration and a reaction time as follows.

‘0.1x’, 20 min

‘1x’, 20 min

‘0.1x’, 10 min

‘1x’, 10 min

‘0.1x’, 1 min

‘1x’, 1 min

Q.1.1. (10 points) First, make an experimental schedule in order to perform all reactions, by describing start (○) and stop (●) signs for each reaction in the table in the Answer Sheet, allowing at least 1 min between the beginning of each reaction. An example for the reaction of '0.1x, 20 min' has been described in the table in the Answer Sheet.

Q.1.2. (15+10 points) Perform the enzymatic reactions according to the protocol described below and the schedule you made in Q.1.1. Use a new pipette tip in every manipulation. Agitate a mixture by tapping a test tube immediately after an addition. After you perform all the reactions, measure A_{400} of the samples. Write the obtained values in the table in the Answer Sheet, and plot them in the graph. **Please note that since water has been used as blank, the line will not pass through 0 (zero) on Y-axis (origin).**

Protocol for measurement of acid phosphatase activity

1） Mix 0.12 ml of 0.5 M Na acetate buffer (pH 5.6) and 0.24 ml of 5 mM pNPP in a test tube. Start the reaction by adding 0.24 ml of an enzyme solution.

2） After the reaction times of 1, 10, and 20 min, respectively, stop the reaction by adding 0.6 ml of 0.5 M NaOH. NaOH stops the reaction and converts the pNP produced into a yellow-colored (A_{400}-absorbing) form.

3） After all reactions are stopped, measure A_{400} of the samples.

Assay of potato acid phosphatase

0.5 M Na acetate buffer(pH 5.6)	0.12	ml
5 mM pNPP	0.24	ml
Enzyme	0.24	ml
0.5 M NaOH	0.6	ml
Sum	1.2	ml

Q.1.3. (15 points) Which enzyme concentration gave better linearity in the relationship between time and A_{400}? Circle the correct one on the Answer Sheet. Read the slope of this straight line from the graph.

Q.1.4. (5 points) Using the slope obtained in Q. 1.3, calculate the activity in the form of A_{400} change per min per 1 ml of an enzyme solution of concentration '1x'. The length of the light path (L) is 1cm. Your answer should be written with your calculations and the appropriate unit in the Answer Sheet.

Q.1.5. (5 points) Convert the absorbance change obtained in Q.1.4 to a concentration change by assuming the ε_{400} of pNP to be 19000 M^{-1} cm^{-1}. Your answer should be written with your calculations and the unit per min per 1 ml of '1x enzyme' solution in the Answer Sheet.

Q.1.6. (5 points) Convert the concentration change obtained in Q.1.5. to a change in number of moles of pNP. Your answer should be written with your calculations in moles per min per ml of '1x enzyme' solution in the Answer Sheet.

Q.1.7. (5 points) Calculate the total activity (in moles per min) in 4 ml of '1x enzyme' solution that was initially given.

Task 2 (30 points)

Protein determination

Protein concentration is determined by using a standard protein such as bovine serum albumin (BSA). In Task 2, you will determine a BSA-equivalent concentration of the 1x enzyme solution by the Bradford method. The Bradford method takes advantage of an increase in absorption of Coommassie Brilliant Blue at 595 nm when it is bound to protein.

By diluting a concentrated BSA solution (0.4 mg protein ml^{-1}) with 3% NaCl, a 1/2-dilution series was made (0.4, 0.2, 0.1, and 0.05 mg protein ml^{-1}). The dilution series of BSA and the 0.1x enzyme solution, which was made in Task 1, were all similarly treated with dye. Optical density at 595 nm (OD_{595}) of these samples was measured and recorded in the table below.

Table

Sample	[BSA] ($mg \cdot ml^{-1}$)	OD_{595}
	0.00	0.000
	0.05	0.070
	0.1	0.143
	0.2	0.261
	0.4	0.521
0.1x enzyme solution		0.180

Optical density (OD), a measure of the extent to which a substance transmits light or the ‘absorbance’ of suspension of particles.

Q.2.1.(10 points) Plot OD_{595} against BSA concentration in the graph in the Answer Sheet and depict an approximate straight line.

Q.2.2.(10 points) Estimate a protein concentration of the 0.1x enzyme solution from the graph, and obtain the protein concentration of the 1x enzyme solution.

Q.2.3.(10 points) Calculate the specific activity (activity per min per mg protein) of the 1x enzyme solution. Your answer should be written with your calculations and the unit per min per mg protein in the Answer Sheet.

STUDENT CODE:

Student Code: _____

20th INTERNATIONAL BIOLOGY OLYMPIAD

12th – 19th July 2009

Tsukuba, JAPAN

PRACTICAL TEST 2

BIOCHEMISTRY

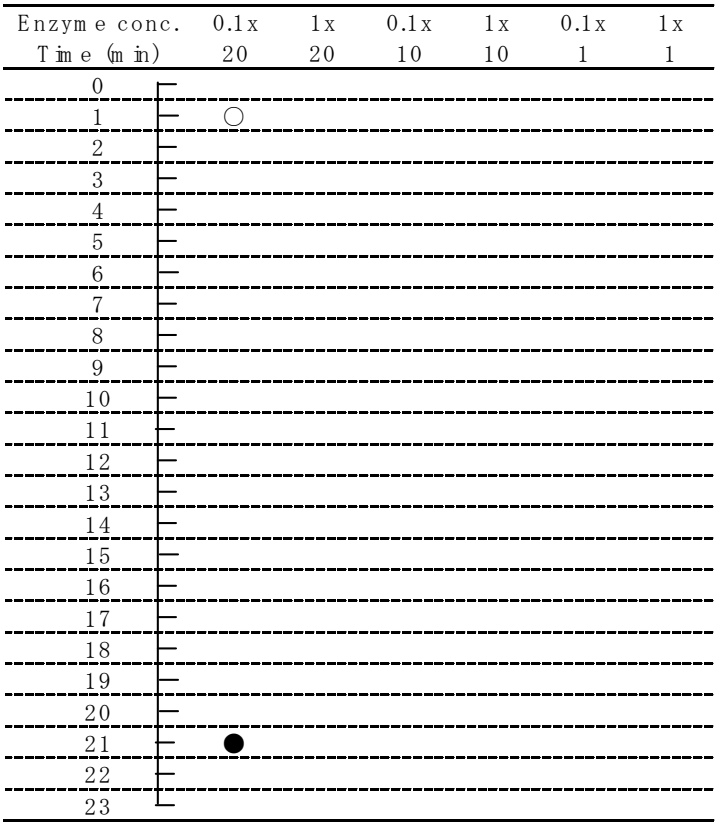
Total Points: 100

Duration: 90 minutes

ANSWER SHEET

STUDENT CODE:

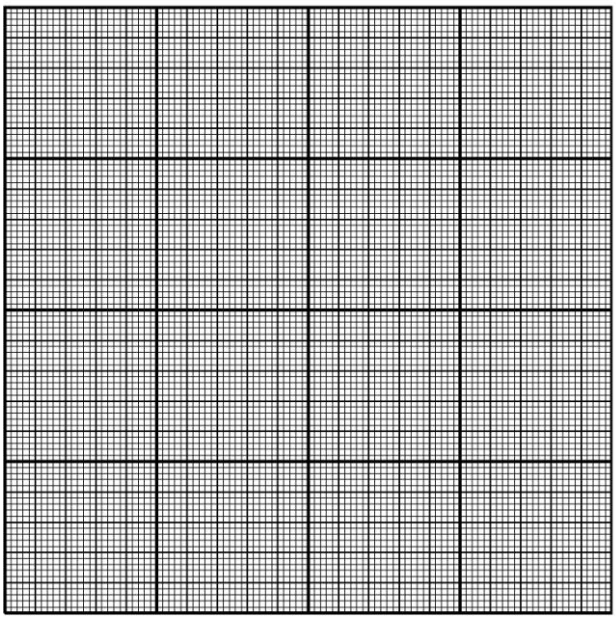
Q.1.1. (10 points)



Q.1.2. (15+10 points)

Time (min)	Enzyme concentration	
	1x	0.1x
1		
10		
20		

STUDENT CODE:



Q.1.3. (15 points)

Linearity : 1x 0.1x

Q.1.4.(5 points)

STUDENT CODE:

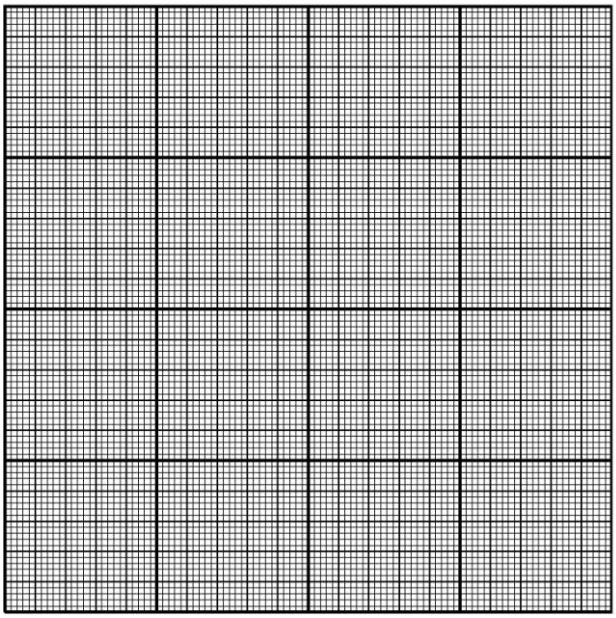
Q.1.5. (5 points)

Q.1.6. (5 points)

Q.1.7. (5 points)

STUDENT CODE:

Q.2.1. (10 points)



Q.2.2. (10 points)

Q.2.3. (10 points)

********** END OF PRACTICAL TEST 2 **********