Country:

Student Code:

27th International Biology Olympiad

17th-23rd July, 2016

Hanoi, Vietnam

Practical Exam 3

BIOCHEMISTRY AND MICROBIOLOGY

Answer Key

Total points: 100

EXPERIMENT 1 (40 points)

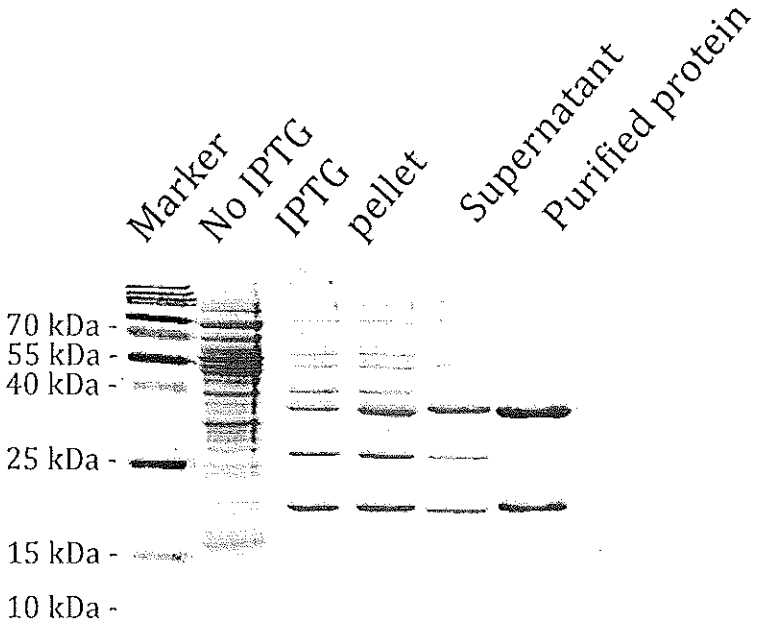
Q.1.1. (6 points)

	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
Sample	Protein Marker	**No_IPTG**	**IPTG**	**Pellet**	**Super**	**Puri-P**
Stock (µl)	5	10.4	17.9	14.9	14.9	15.0
H_2O (µl)	5	14.8	11.0 - 11.1	12.5	12.5	12.5
2X SDS-PAGE loading buffer (µl)	10	14.8	11.0 - 11.1	12.6	12.6	12.5
Total volume (µl)	20	40	40	40	40	40

0.5 point for each correct answer for Stock.

0.4 point for each correct answer for H_2O and loading buffer.

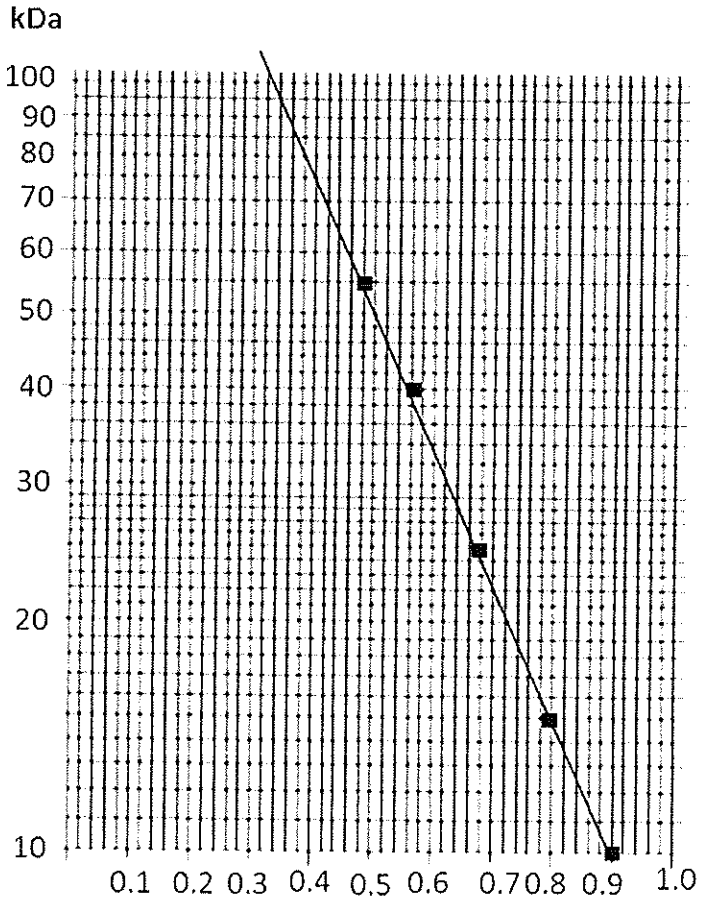
Q.1.2. (10 points)



5 points for SDS-PAGE picture with bands of sample.

Add 1 point for clearly visible bands in each well except Marker.

Q.1.3. (4 points)



2 points for at least 5 correct spots on the graph.

2 points for the linear curve.

Q.1.4. (4 points)

Protein	Size (kDa)
H	20 ± 2
B	35 ± 2

Scoring bases on the student's answer in Q.1.3.

2 points for each correct answer.

Q.1.5. (4 points)

Statement	True	False
A. H protein is over expressed in LB media with IPTG.	✓	
B. B protein is completely soluble in the nickel binding buffer.		✓

C. H and B protein interact with each other.	✓	
D. Majority of recombinant proteins bound to the nickel column.	✓	

4 points for 4 correct answers.

2 points for 3 correct answers.

1 points for 2 correct answers.

0 points for 0-1 correct answers.

Q.1.6. (4 points)

Statement	True	False
A. *Sal*I and *Bam*HI can be used to insert *b* gene into MCS-1	✓	
B. Gene *h* and *b* should be cloned in the same orientation to be expressed simultaneously.	✓	
C. Gene *h* and *b* should be in the same reading frame to be expressed simultaneously.		✓
D. To maintain the plasmid, ampicillins should be added to the culture medium.	✓	

4 points for 4 correct answers.

2 points for 3 correct answers.

1 points for 2 correct answers.

0 points for 0-1 correct answers.

Q.1.7. (4 points)

Sample	Size (kDa)
The peak observed in sample 1	50 ± 10
The peak observed in sample 2	100 ± 20

Q.1.8. (4 points)

Statement	True	False
A. H protein exists as monomer.		✓
B. H and B probably exist as heterodimer.		✓

C. H protein helps to stabilize B protein	✓	
D. In native gel-filtration column analysis, retention time of a protein is proportional to its molecular weight.		✓

Scoring bases on the student's answer in Q.1.7.

4 points for 4 correct answers.

2 points for 3 correct answers.

1 points for 2 correct answers.

0 points for 0-1 correct answers.

EXPERIMENT 2. (30 points)

Q.2.1. (4 points)

Ascorbic acid sample				Coffee extract sample			
Diluted solution	Volume (µl) of solution used for dilution	Volume of H_2O (µl)	Concentration (mg/ml)	Diluted Solution	Volume (µl) of solution used for dilution	Volume of H_2O (µl)	Concentration (mg/ml)
AA1	40	160	0.2	CC1	200	0	5
AA2	100	100	0.1	CC2	100	100	2.5
AA3	100	100	0.05	CC3	100	100	1.25
AA4	100	100	0.025	CC4	100	100	0.625

0.25 point for each correct answer.

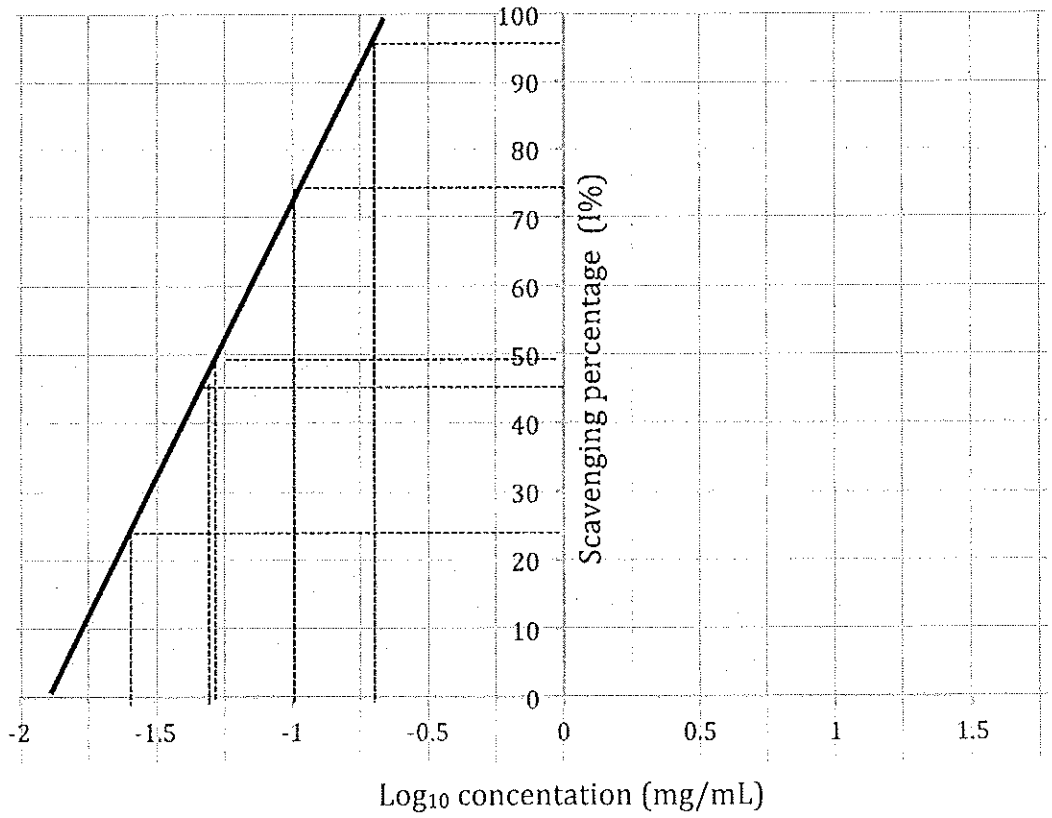
Q.2.2. (5 points)

Ascorbic acid				Coffee extract			
Solution	Log_{10} of concentration (mg/mL)	Mean absorbance	SC%	Solution	Log_{10} of concentration (mg/mL)	Mean absorbance	SC%
Control		0.90 ± 0.01					
AA1	- 0.70	0.076 ± 0.007	91.48 ± 9.1	CC1	0.70	0.225 ± 0.022	75.01± 7.5
AA2	- 1	0.249 ± 0.024	72.34 ± 7.2	CC2	0.40	0.334 ± 0.033	62.88 ± 6.2
AA3	- 1.30	0.511 ± 0.051	43.15 ± 4.3	CC3	0.10	0.602 ± 0.060	33.06 ± 3.3
AA4	- 1.60	0.716 ± 0.071	28.10 ± 2.8	CC4	- 0.20	0.744 ± 0.074	17.26 ± 1.7

0.2 point for each correct answer.

Scoring values of SC% based on the value of mean absorbance.

Q.2.3. (5 points)



3 points for 4 correct spots on the graph.

2 points for the linear curve.

Q.2.4. (5 points)

For example: SC_{50} of ascorbic acid

$Log(SC_{50}) = -1.23$

$SC_{50} = 10^{-1.23} = 0.058$

Ascorbic acid	Coffee extract
0.06 ± 0.006	2.01 ± 0.20

Scoring values of SC_{50} based on the graph.

Q.2.5. (3 points)

Z	>	Y	>	X

Q.2.6. (4 points)

Statement	True	False
A. Antioxidant activities of the diluted coffee extracts are negligible.		✓
B. To obtain more accurate determination of antioxidant activities, another experiment with higher concentration samples needs to be carried out.		✓
C. Activity of antioxidant enzymes in coffee extract resulted in the above result.		✓
D. If NADH is added in the wells, there will be no change in the assumed absorbance	√	

4 points for 4 correct answers.

2 points for 3 correct answers.

1 points for 2 correct answers.

0 points for 0-1 correct answers.

Q.2.7. (4 points)

Statement	True	False
A. The student has used DPPH 0.1 mM for Protocol A*.		✓
B. The student has loaded 10 μl of sample in each well for Protocol A*.	√	
C. After adding DPPH, the student has incubated the 96-well for a shorter time than in Protocol A.	√	
D. The student has used better solvent for antioxidants.		✓

4 points for 4 correct answers.

2 points for 3 correct answers.

1 points for 2 correct answers.

0 points for 0-1 correct answers.

EXPERIMENT 3. (30 points)

Q.3.1. (3 points) 2 ± 0.1 h

Q.3.2. (3 points) $4.2\times2\times10^8\times2^3 = 67.2\times10^8$ cells

Q.3.3. (2 points)

Cultivation time (h)	Sample	Dilution factor	Sample volume (mL)	Deionized water volume (mL)	Total volume (mL)
0	A0	2	15	15	30
6	A2	5	6	24	30
9	A3	10	3	27	30
15	A5	20	1.5	28.5	30

0.25 point for each correct answer.

Q.3.4. (9 points)

Cultivation time (h)	Sample	Volume of 0.1 M NaOH titrated (First time, mL)	Volume of 0.1 M NaOH titrated (Second time, mL)	Volume of 0.1 M NaOH titrated (Average, mL)
0	A0	3.8 ± 0.1	3.8 ± 0.1	3.8 ± 0.1
6	A2	2.2 ± 0.05	2.2 ± 0.05	2.2 ± 0.05
9	A3	2.4 ± 0.05	2.4 ± 0.05	2.4 ± 0.05
15	A5	2.7 ± 0.05	2.7 ± 0.05	2.7 ± 0.05

0.75 point for each correct answer.

Q.3.5. (10 points)

Cultivation time (h)	Sample	Volume of 0.1M NaOH (mL) need to be used to titrate 30 mL of stock sample	Lactic acid concentration (g/L)
0	A0	7.6 ± 0.2	0
6	A2	11 ± 0.25	(A2-A0)*9/30 = 1.02
9	A3	24 ± 0.5	(A3-A0)*9/30 = 4.92
15	A5	54 ± 1	(A5-A0)*9/30 = 13.92

*1 point for each correct answer for Volume of 0.1 M NaOH (scoring based on the result of **Q.3.4**).*

2 points for each correct answer for lactic acid concentration.

Q.3.8 (3 points) $0.33x2x10^8 + 6x2x10^8 = 12.66\times10^8$ tế bào.