

私立臺北醫學院 90 學年度第 2 學期 期 中 考 試 (命 試) 題 紙

系 級	科 目	授 課 教 師	考 試 日 期	學 號	姓 名
醫二	生化實驗	陳運初	91年6月11日第 節		

※①請注意本試題共 4 張。如發現頁數不足及空白頁或缺印，應當場請求補齊，否則缺少部份概以零分計。
 ②每張試題卷務必填寫(學號)、(姓名)。

1. 請問如何利用下列所列方法中區分糖水溶液中含的是 Fructose 或是 Glucose, Why? (Molish test; Picric acid test; Benedict test; Seliwanof's test; Bile test; Iodine test) (8%)
2. 請問在本學期實驗中測定 Urease 活性的原理是利用什麼方法，請簡述之，以及如何測定出一個酵素的最佳反應溫度?(12%)
3. 請問在抽取 Plasmid DNA 時，solution I 中含有 EDTA 其主要功能為何? DNA 經 agarose gel 分離如何觀察它?(8%)
4. 請問在 western blot 實驗中蛋白質由 SDS-page 將其轉漬到 nitrocellulose membrane (NC 膜)其目的為何? 又何謂一級抗體(1st antibody)及二級抗體(2nd antibody)其意義各為何?(12%)

系級	科目	授課教師	考試日期	學號	姓名
	醫學系生化實驗	黃考華	91年6月11日第 _____ 節		

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Gel filtration (10 points)

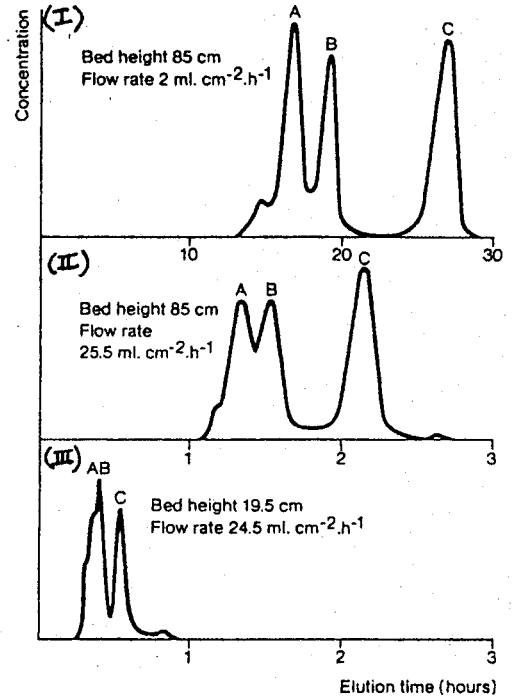
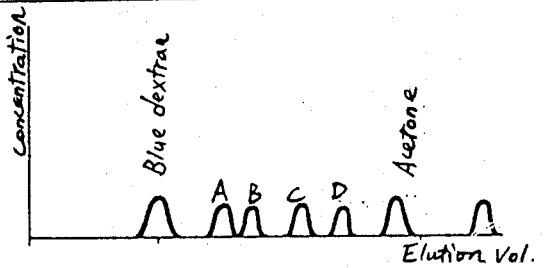
1. According to the pattern of gel filtration, please give the definition of (A) V_0 (B) V_t (C) V_e (D) K_{av} (2 points)

- (A) V_0 :
- (B) V_t :
- (C) V_e :
- (D) K_{av} :

2. According to the gel filtration experimental condition as described,

- (A). The molecular weight of the A,B,C protein is _____ > _____ > _____. (2 points)
- (B). Please describe the (I),(II), (III) experimental condition priority? _____ is better than _____ than _____. (1 point)
- (C) Why? Please describe the scientific reasons in detail. (2 points)

(D) Describe the application of gel filtration? (3 points)

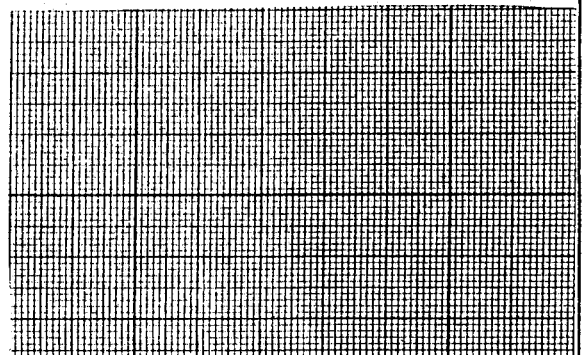


Protein quantification (5 points)

(A). Please describe the principle of Bradford method? (1 point)

(B). Please estimate the protein concentration ($\mu\text{g/ml}$) of the unknown sample: (4 points)

BSA Standard ($\mu\text{g/ml}$)	OD 595 nm
2	0.147
4	0.292
6	0.424
8	0.528
10	0.652
Unknown sample	0.341



Lipid peroxidation (Total 5 points, 1 point each)

- (A) Please give the chemical equation of Fenton reaction: _____
- (B) What will be the final product of Arachidonic acid in lipid peroxidation? _____
- (C) What chemical compound do we use to identify the final product of lipid peroxidation? _____, and which color will present? _____
- (D) Why we could use TEP (tetraethoxypropane) to make the standard curve for estimating the concentration of the final product of lipid peroxidation? _____

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Part I:

In the experiment of polymerase chain reaction (PCR), please match the choices.

- A. *Tag* polymerase
- B. 72 °C
- C. 94 °C
- D. 55 °C
- E. primers
- () Annealing temperature
- () Denature temperature
- () Polymerization temperature
- () A pairs of single strand DNA
- () An enzyme

Part II:

- () Identified the correct statement for plasmid DNA. (1) an extrachromosomal DNA (2) with super coil from (3) with open circular from (4) all correct above
- () Which one does not include in the protocols of *E. coli* transformation (1) 42 °C, 2 min (2) on ice 30-40 min (3) add solution II (4) plating the competent cells on AP-plate.
- () In the "transformation" experiment, the transformed *E. coli* was (1) ampicillin (2) tetracyclin (3) neomycin (4) streptomycin resistance
- () After electrophoresis of DNA, the agarose gel was stained with (1) coomassie blue (2) blur dextrane (3) ethidium bromide (4) methyl orange
- () If you want to pipette 150µl solution, which micropipette is your best choice (1) 20p (2) 200p (3) 1000p (4) 5000p
- () The optimum reaction temperature for most restriction enzyme was (1) 4 °C (2) 37 °C (3) 42 °C (4) 72 °C
- () In our experiment, the direction of DNA electrophoresis was from (1) anode to cathode (2) cathode to anode.
- () Which chemical compound does not used in SDS-PAGE? (1) SDS (2) acrylamide (3) bisacrylamide (4) TEMED
- () Which compound is not one of the component of sampling buffer (SDS-PAGE experiment)? (1) SDS (2) acrylamide (3) β-mercaptoethanol (4) Tris
- () Which dye was used to stain SAS-PAGE? (1) EtBr (2) coomassie blue (3) methylene blue (4) methyl orange
- () The SDS-PAGE used in the bio-experimental class is not a (1) slab (2) continuous (3) denature (4) polyacrylamide gel system
- () The *Hind*III is a type (1) I (2) II (3) III (4) IV restriction enzyme.
- () The substrate of restriction enzyme is (1) DNA (2) RNA (3) protein
- () Which method can transfer DNA into eucaryote as well as prokaryote ? (1) transformation (2) transfection (3) transfusion (4) electoporation
- () Which statement is incorrect? (1) *Hind*III digested λ DNA can be used as a DNA marker (2) CaCl₂ can change the net charge of *E. coli* cell membrane from positive to negative while preparation of competent cells. (3) *Tag* polymerase has no ability of proofreading. (4) β-mercaptoethanol is a reducing agent which was used to disrupt disulfide bond of protein.

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Part III: Please answer Y (yes) or N (no)

- () Before agarose gel staining, you must wear plastic gloves.
- () In PCR experiment, the mineral oil is used to prevent H₂O evaporation.
- () Mg⁺⁺ ion is a cofactor of EcoRI restriction enzyme and Taq polymerase
- () In extraction of plasmid DNA experiment, you should discard the supernatant after addition of solution I, II, III and centrifugation.
- () "Molecular sieving" is a major principle of separation in agarose gel and SDS-PAGE.