**BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI**

**II SEMESTER: 2013-14**

**COMPREHENSIVE EXAMINATION**

**INSTRUMENTAL METHODS OF ANALYSIS BIO C391/BIO F244**

**Time: 2h**

**Maximum Marks: 60 Date: 15/4/2014**

**Note:** It is a **2 hour** question paper in which maximum 30 minutes is allotted for part A and remaining time for part B& C. Part A (20 marks) should be answered in the answer sheet provided alongside and submitted before collecting Part B (5 marks) & C (35 marks) . Part B & and Part C should be answered in separate answer sheets.

**PART A**

Q1.Fill in the blanks (8)

1. Ionic protein molecules do not have any net charge at their \_\_\_\_\_\_\_\_\_\_\_\_\_.
2. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ is a technique used for selective separation of ionic analytes that are separated based on mobility.
3. Polyacrylamide gels are made by cross linking acrylamide with N, N’-methylenebisacrylamide and the cross linking reagent used is \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
4. The type of separation technique in which instead of constant current, alternate long pulses of current in forward direction with shorter pulses in either opposite or sideways direction is called \_\_\_\_\_\_\_\_\_\_\_\_\_.
5. \_\_\_\_\_\_\_\_\_\_\_\_\_\_ is the process by which the solution of sample is introduced through an orifice into a high velocity gas jet.
6. A dye suspended in thin film of gelatin sandwiched between glass plates can be used as \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_for spectrometry.
7. 1200-1400 groves /mm are used for preparation of diffraction gratings of

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ range of light.

1. Cuvettes to be used in UV region are made of \_\_\_\_\_\_\_\_\_\_\_.

**PTO**

Q2. Write the most appropriate answer sheet. Use only capital letters. One mark will be given for each correct answer. Also 0.25 marks will be deducted for each incorrect answer. (12 marks)

1. What is the most suitable melting temperature for the following PCR primer:

5'-AATCCAGGTATTCGCGAAG-3'?

a. 66 C

b. 56 C

c. 40 C

d. 38 C

e. none of above

2. The types of deviation in Beer’s Lambert’s caused due to broad band width of radiation is

a. real deviations

b. chemical Deviations

c. instrumental Deviations

d. none of the above

3. Heat stable DNA polymerases are used for PCR because

a. they are able to denature DNA at high temperature

b. heat sensitive DNA polymerases cannot extend DNA primers

c. they can start DNA replication without the help of primers

d. they allow the reaction to be automated

e. they are able to make DNA in both the 3’ to 5’ and the 5’ to 3’ directions

4. You wish to amplify a 22,345 base-pair region of mouse DNA using the polymerase chain reaction. You design a pair of primers that are 20 and 22 bases in length (respectively) and have identical melting temperatures. However, when you run your reaction it fails. This is most likely because:

a. your primers are not the same length

b. the region of DNA you are attempting to target is too large

c. your primers are too short

d. you targeted mouse DNA; only human DNA can be used for PCR

e. you used more than one primer in the reaction

5. The reverse transcriptase reaction can be primed by

a. target sequence specific primers

b. random hexamers

c. oligo dT primers

d. all of the above

e. none of a, b and c

6. The nuclease activity of Taq DNA polymerase is specifically for

a. 5' to 3'

b. 3' to 5'

c. none of the above

d. both a and b

e. both endo and exo nuclease activity

7. Which of the following hybridize with the ends of the gene to be amplified in a PCR reaction?

a. Taq polymerase

b. Deoxyribonucleotides

c. Ribonucleotides

d. RNA primer

e. DNA primers

8. Which of the following is not included in a typical PCR reaction

a. dNTP

b. Magnesium chloride

c. DNA primers

d. E. Coli DNA polymerase

e. all of above are generally added

9. Which of the following is an application of PCR technology?

a. epidemiology

b. forensic science

c. gene mapping

d. *in vitro* mutagenesis

e. all of above

10. *Taq* polymerase is a commonly used enzyme in PCR because this enzyme is

a. not prone to errors

b. have both 5' to 3' and 3' to 5' nuclease activity

c. a faster polymerase

d. can withstand low temperatures

e. thermostable

11. The purpose of washing in an ELISA experiment was to

a. clean off the wells.

b. dilute the antibody solution added

c. rinse away non-specific binding

d. keep the slide from drying out.

e. none of above

12. The primary reason to block with BSA/milk in Sandwich ELISA experiment is to

a. fix the antigen-antibody reaction

b. to increase binding affinity of secondary antibody

c. to reduce non-specific binding of antigen

d. to reduce non-specific binding of coating antibody

e. HRP tagged antibodies bind only when a blocking step is done

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**Answer Sheet for Part A**

Q1.

1. \_\_\_\_\_\_\_\_\_\_\_\_
2. \_\_\_\_\_\_\_\_\_\_\_\_
3. \_\_\_\_\_\_\_\_\_\_\_\_
4. \_\_\_\_\_\_\_\_\_\_\_\_
5. \_\_\_\_\_\_\_\_\_\_\_\_
6. \_\_\_\_\_\_\_\_\_\_\_\_
7. \_\_\_\_\_\_\_\_\_\_\_\_
8. \_\_\_\_\_\_\_\_\_\_\_\_

Total of Q1. =

Q2. 1.\_\_\_\_ 2.\_\_\_\_ 3.\_\_\_\_\_ 4.\_\_\_\_\_

5. \_\_\_\_ 6. \_\_\_\_ 7. \_\_\_\_\_\_ 8.\_\_\_\_\_\_

9. \_\_\_\_\_ 10.­­\_\_\_\_\_ 11. \_\_\_\_\_ 12. \_\_\_\_\_

Correct answers marks =

Incorrect Answers marks =

Total of Q2.=

Total of part A =

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**PART B**

Q3. A GLC column was operated under the following conditions: (5)

* **column:** 1.10 m × 2.0 mm, packed with Chromosorb P; weight of stationary liquid added, 1.40; density of liquid, 1.02 g/mL
* **measured outlet flow rate:** 25.3 mL/min
* **temperature:** room, 21.2 oC; column, 102.0 oC
* **retention times:** air, 18.0 s; methyl acetate (MA), 1.98 min; methyl propionate (MP), 4.16 min; methyl *n*-butyrate (MB), 7.93 min.
* **peak widths at base:** 0.19, 0.39 and 0.79 respectively

Calculate the following (use the retention time in minutes):

(a) α values for each adjacent pair of compounds

(b) the resolution for each pair of compounds

**PART C**

Q4 (a) What is the purpose of standard curve in an ELISA experiment? (2)

(b) Mention 5 applications of AAS. (2.5)

Q5 (a) What is Steady State Electrophoresis. Briefly explain. (4)

(b)Which temperature range would be preferred for PAGE-warm, normal or cold? Why? (4)

Q6 Draw a schematic diagram representing the following (6)

1. Flame photometer
2. Single beam spectrophotometer

Q7.(a) What are the components present in an AAS but absent in a flame photometer, What is their function? (3)

(b)Arrange in decreasing order of wave length. (1)

Q8(a). Does refractive index of solvent effect the Beer Lamberts Law? Justify your

answer. (2)

(b) Enumerate the 5 major components of a monochromator. (2.5)

Q9. (a) Differentiate between the method to make master grating and replica grating. (2)

(b) Briefly describe the functioning of photovoltaic cell. What are the disadvantages of its use? (4)

Q10. What is electrophoretic mobility? Enumerate 5 factors affecting it. (3)

**THE END**