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

**Second Semester, 2012-2013**

**BIO C391/ BIO F 244 Instrumental Methods of Analysis**

**Comprehensive Exam (Close Book)**

**Date: 04.05.13 MM: 45 Time: 2H**

* The question paper contains five questions in all. Answer all parts of each question together.
* Answer should be precise and to the point.

---------------------------------------------------------------------------------------------------------------------

**Q1a.** An analyte solution has molar absorptivity of **8.2** x104 L mol-1 cm-1 at 470 nm. Calculate the absorbance and %T in (i) 1.0 and (ii) 2.0 cm cells if the concentration of the solution is 4.2 x 10-6 M. (Show the calculation clearly). **(4)**

**Q1b.** What are the basic components used in UV-Visible spectrophotometer and what is the function of each? Give an application of each UV-visible spectroscopy technique used in Molecular and Micro Biology. **(4)**

**Q2a.** What is Sandwich ELISA and how is it different from Indirect ELISA? **(3)**

**Q2b.** If the primary antibody was raised in mouse and there are four commercially HRP-linked secondary antibodies available namely Donkey anti-rabbit, Goat anti-mouse, Mouse anti-rat and Rat anti-rabbit secondary antibody. Which one is best for indirect ELISA and why? Explain. **(4)**

**Q3.** What is the difference between Native-PAGE and SDS-PAGE? Explain the mechanism of separation of proteins in SDS-PAGE. **(6)**

**Q4a. Match the following. (4)**

|  |  |  |  |
| --- | --- | --- | --- |
| 1. | UV-Visible | A | promotes ejection of electrons |
| 2. | Bromophenol blue | B | breaks up liquid into small droplets |
| 3. | X-ray | C | removes large droplets from stream |
| 4. | nebulizer | D | Detecting dye |
| 5. | aerosol modifier | E | due to collision between excited molecules |
| 6. | self quenching | F | promote electrons to higher orbital |
| 7. | self absorption | G | Tracking dye |
| 8. | Coomassie Brillant Blue | H | due to overlapping of the wavelengths of emission and absorption peak |

**Q4b.** Appropriately define and differentiate between enantiomers and diastereomers. What would be the optical purity of a sample F, whose specific rotation is +50 °(D) and -50° (L), and its mixture has specific rotation of -35°(L)? Show calculations. **(4)**

**Q4c.** How do the temperature, solvent viscosity, chemical structure of molecule and pH affect fluorescence? **(4)**

**Q5a.** Desolvation, volatilization and dissociation terms are used in some techniques. Name the techniques and explain the whole process where these terms are applied with reference to techniques. **(4)**

**Q5b.** Taq and Pfu DNA polymerase are commonly used enzymes for PCR. What is the specificity of these enzymes? Is it possible to find short tandem repeat polymorphism through PCR? Explain. **(4)**

**Q5c.** What ispartition chromatography? Explain. What would you infer about the relative mobility of two compounds X and Y in TLC, where the Kd of X is less than that of Y?  **(4)**