Subject Code : P8BC2

CORE COURSE II – ANALYTICAL TECHNIQUES

Unit 1 Spectroscopic techniques

Laws of absorption and absorption spectrum. Principle, instrumentation and applications of UVvisible spectrophotometry and spectrofluorimetry. Basic principles of turbidimetry and nephelometry. Principle, instrumentation and applications of luminometry. Atomic spectroscopy – principle and applications of atomic flame and flameless spectrophotometry. Use of lasers for spectroscopy. - MALOF TOF.

Unit 2 Centrifugation and radioisotope techniques

Ultracentrifuges– Analytical ultracentrifuge– instrumentation and applications. Preparative ultracentrifuge– types, instrumentation and applications of preparative rotors. Analysis of subcellular fractions and determination of relative molecular mass– sedimentation velocity and sedimentation equilibrium.

Units of radioactivity. Detection and measurement of radioactivity– solid and liquid scintillation counting, scintillation cocktails and sample preparation. Cerenkov counting. Autoradiography. Applications of radioisotopes in biology. Radiation hazards.

Unit 3 Electrophoresis and Electrochemical techniques

Electrophoresis: General principles. Support media. Electrophoresis of proteins– SDS-PAGE, native gels, gradient gels, isoelectric focusing, 2-DPAGE. Cellulose acetate electrophoresis. Detection, estimation and recovery of proteins in gels. Electrophoresis of nucleic acids– agarose gel electrophoresis, DNA sequencing gels, pulsed field gel electrophoresis. Membrane blotting and hybridization of nucleic acids– Southern, Western, dot-blot and fluorescent in situ hybridization. RFLP- technique and applications.

Unit 4 Chromatography

Principle, instrumentation and applications of thin layer and gas chromatography. Column chromatography-packing, loading, eluting and detection. Ion-exchange chromatographypreparation of resins, procedure and applications. Chromatofocusing. Molecular exclusion chromatography-principle, gel preparation, operation and applications. Affinity chromatography– principle, materials, procedure and applications. Special forms of affinity chromatography– immunoaffinity, metal chelate, dye-ligand and covalent chromatography. HPLC– principle, materials, instrumentation and applications. Capillary electrochromatography.

Unit 5 PCR technique:

PCR– basic principle, RT-PCR, quantitative PCR and in situ PCR. Diagnostic and laboratory applications of PCR. Comet assay. Monitoring of oncogenes and antioncogenes. Mutagenecity testing– Ame's test. DNA finger printing, DNA foot printing.

Books recommended

1. Wilson and Walker. A biologists guide to principles and techniques of practical biochemistry. 5th ed. Cambridge University Press 2000.
2. Boyer, R. Modern Experimental Biochemistry. 3rd ed. Addison Weslery Longman, 2000.
3. Upadhyay, Upadhyay and Nath. Biophysical Chemistry Principles and Techniques. Himalaya Publ. 1997.
4. Simpson CFA & Whittacker, M. Electrophoretic techniques.
5. Sambrook. Molecular Cloning. Cold Spring Harbor Laboratory, 2001.
6. Friefelder and Friefelder. Physical Biochemistry – Applications to Biochemistry and Molecular Biology. WH Freeman & Co. 1994.
7. Pavia et al. Introduction to Spectroscopy. 3rd ed. Brooks/Cole Pub Co., 2000.