ST.JOSEPH'S COLLEGE FOR WOMEN (AUTONOMOUS), VISAKHAPATNAM

VII SEMESTER **BIOTECHNOLOGY** TIME: 3 Hrs/Week

BTH 7702 (3) **BIOPHYSICAL AND BIOCHEMICAL TECHNIQUES**

W.e.f 20AH Batch (Core course) Max. Marks: 100

OBJECTIVES: To enable the students to –

- Acknowledge the different isolation methods for screening the macromolecules.
- Know the principles and applications of various microscopes.
- Get the comprehensive idea about separation techniques
- Be able to characterize the biomolecules by different spectroscopic techniques.
- Get the different radioisotope tracer techniques and their role in biology.

I. Learning outcomes:

1. Learnt about various isolation techniques for isolation and concentration of macromolecules. They will also understand the principles and applications of different Microscopes
2. Understand the techniques of chromatography, centrifugation and electrophoresis
3. Achieve a basic understanding of characterization of biomolecules by different Spectroscopic techniques
4. Familiarize with the various radioisotope tracer techniques and their role in biology. Eventually they learn safety measures in handling radio-isotopes.

UNIT-I: Isolation techniques

1. Cell disruption techniques - sonication, french press, enzymatic, non-enzymatic techniques.
2. Isolation of proteins –a. salting in/out, ammonium sulphatefractionation.
   Nucleic acids –b. polar solvents precipitation.
   Lipids –c. extraction by differential solubility.

3. Concentration of macromolecules: flash evaporation, lyophilisation, pressure dialysis, reverse dialysis, hollow fiber membrane filters and reverse osmosis.

UNIT – II: Microscopic studies

1. Principles and applications: Light, compound, phase contrast, confocal and SEM and TEM.

UNIT – III: Separation techniques

1. Principles and applications of gel-filtration, ion-exchange and affinity chromatography.TLC, GLC and HPLC.
2. Basic principles of sedimentation, Types of centrifuges –Micro-centrifuge, High speed & Ultracentrifuges; Preparative centrifugation; Differential & density gradient centrifugation; Applications (Isolation of cell components); Analytical centrifugation.
3. Determination of molecular weight by sedimentation velocity & sedimentation equilibrium methods.
4. General principles of electrophoretic techniques. Poly Acryl amide Gel Electrophoresis (PAGE), Isoelectric focusing.Isotachophoresis.2-D Electrophoresis.Capillaryelectrophoresis. Agarose gel electrophoresis of DNA and RNA.

5. Blotting techniques.
6. DNA fingerprinting.

UNIT – IV: Spectroscopy

1. Electromagnetic spectrum of light.
2. Principles, instrumentation and applications of UV-Visible, infrared, Raman, fluorescence, flame photometry, atomic absorption, plasma emission, ESR, ORD, CD, NMR spectroscopy.
3. Spectro-fluorimetry and mass spectrometry, X-ray diffraction.
4. Flow cytometry.

UNIT V: Radioisotope tracer techniques

1. Nature and types of radioactivity, Preparation of labelled biological compounds.
2. Labeling of carbohydrates (C^{14} acetate), proteins (S^{35} methionine, I^{125}aminoacid) and nucleic acids (P^{32} dATP).
3. Detection and measurement of radioactivity. Autoradiography.
4. Biological uses of radioisotopes, Safety guidelines.

REFERENCES

1. D. Holme& H. Peck, Analytical Biochemistry, 3rd Edition, Longman, 1998.
2. Freifelder D., Physical Biochemistry, Application to Biochemistry and Molecular Biology, 2nd Edition, W.H. Freeman & Company, San Fransisco, 1982.
3. Keith Wilson and John Walker, Principles and Techniques of Practical Biochemistry, 5th Edition, Cambridge University Press, 2000.
4. Biophysical chemistry principles and techniques by Upadyay, Upadyay and Nath (Himalaya publishing).

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