Universitat Autònoma de Barcelona

2018/2019

Instrumental Techniques

Code: 100998
ECTS Credits: 6

Degree	Type	Year	Semester
2500502 Microbiology	FB	2	1

Contact

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Use of languages

Principal working language: catalan (cat)
Some groups entirely in English: No
Some groups entirely in Catalan: No
Some groups entirely in Spanish: No

Prerequisites

It is recommended to review the basic concepts of Biochemistry of first year, especially the physical-chemical characteristics of macromolecules.

It is recommended to take this course at the same time as "Laboratori Integrat III" (100978) of this degree.

Objectives and Contextualisation

MODULE II: INSTRUMENTAL TECHNIQUES IN BIOCHEMISTRY

The general objective is the students learn the instrumental techniques that are developed in a laboratory and that they may need throughout their studies and professional activity.

This objective can be specified in:

-Acquire and understand the theoretical basis of the main instrumental techniques
-Application of these techniques in the field of microbiology
-Strengthen the self-learning ability of the student. The student must learn to obtain information and acquire the habit of using this information critically.
-Increase student interest in the technical aspect of science.

Content

Module II: INSTRUMENTAL TECHNIQUES IN BIOCHEMISTRY

Unit 1: Basic Principles of absorption spectroscopy. Lambert-Beer Law. Spectrophotometers. Spectroscopic analysis of biopolymers. Fundamentals of spectrofluorimetry. Spectrofluorimeter. Applications.

Unit 2: Centrifugation. Fundamentals.Sedimentation Coefficient. Factors on which the sedimentation coefficient depends. Instrumentation: preparative and analytical ultracentrifuge. Preparative centrifuge rotors: floating, angular, vertical. Cell fractionation by centrifugation. Centrifugation with density gradients.

Unit 3. Chromatographic techniques. Introduction. Fundamentals and characteristics. Chromatography type: gel filtration, ion exchange, hydrophobic, affinity. High Performance Liquid Chromatography (HPLC). Gas chromatography.

Unit 4: Purification Strategies of macromolecules. Stages of purification. Optimization of each stage. Preparative techniques of nucleic acids: plasmid DNA, bacteriophage DNA, genomic DNA, total RNA and messenger RNA.

Unit 5: Electrophoretic techniques. Protein Electrophoresis: SDS-PAGE, Two-dimensional gel electrophoresis, native electrophoresis. Nucleic acid electrophoresis: native, denaturing, pulsating field, thermal gradient, electroelution.

Unit 6. Hybridization techniques: Western-blot, Southern-blot, Northern-blot, Southwesthern, Microarrays, FISH, in situ hybridization. Labeling techniques.

Unit 7: Polymerase Chain Reaction: PCR. Fundamentals of the technique. Primers design . Set up of the reaction. Applications. RT PCR and Real time PCR.

Unit 8: Recombinant DNA technology. General scheme of a cloning. Types of vectors Expression of recombinant proteins. Libraries for genomic sequencing: concept of representativeness. Libraries of c-DNA versus RNA-seq. Other DNA manipulation techniques (gene editing: CRISPR-cas9)

Unit 9. Mass spectrometry. Calculation of molecular weight by mass spectrometry. Techniques for biopolymers.

Unit 10: Radioactive isotopes. Kinetics of disintegration. Isotopes used in Biochemistry. In vivo labeling. Methods for LabelingNucleic Acids and Protein. Detection & Measurement of Radioactivity. Geiger Mueller (GM) Detectors. Scintillation counters. Autoradiography. Alternative methods to autoradiography (phosphorimaging). Protection in the use of radioactive isotopes. Chemiluminescence systems as an alternative to radioactive methods.

Unit 11: Immunological techniques. Preparation of monoclonal and polyclonal antibodies. Antigen-antibody reaction. Immunoelectrophoresis. Immunoprecipitation. ChIP. RIA. ELISA.

Unit 12: Electron microscope (TEM / SEM). Sample preparation methods.