

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.

Competences

- Apply suitable methodologies to isolate, analyse, observe, cultivate, identify and conserve microorganisms.
- Identify and solve problems.
- Use molecular and immunological techniques in the characterisation of microorganisms and materials of biological origin.

Learning Outcomes

1. Assimilate the basic principles of mass spectrometry and its applications.
2. Identify and solve problems.

3. Identify culture media, culture types and the separation and cloning of eukaryotic cells and the generation of monoclonal antibodies
4. Identify suitable techniques for detecting, quantifying and purifying biological molecules and for determining protein structure.
5. Identify the principles and the sample preparation methods in electron microscopy.
6. Know the functioning of the equipment used in microscopy, chromatography, filtering, dialysis, cytometry, spectroscopy, electrophoresis, amplification and DNA sequencing, among others.
7. Know the principles behind techniques that are used to analyse components of cells, viruses and microbial products.

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, Southwestern, 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 Labeling Nucleic 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.

Methodology

Teaching methodology includes theory classes (30 h), problem classes (12 h), seminar classes (3 h)

Activities

Title	Hours	ECTS	Learning Outcomes
Type: Directed			
Magisterial class	30	1.2	1, 6, 7, 3, 5, 4
Problem Class	12	0.48	1, 6, 7, 3, 5, 2, 4
Seminars Class	3	0.12	1, 6, 7, 3, 5, 2, 4
Type: Supervised			
Group and individual tutoring about seminars and problems	10	0.4	1, 6, 7, 3, 5, 2, 4
Type: Autonomous			
Autonomous Study	62	2.48	1, 6, 7, 3, 5, 2, 4
Problem resolution	14	0.56	1, 6, 7, 3, 5, 2, 4
Work redaction (seminar)	9	0.36	3, 5

Assessment

1. Theory (60%)

1.1 Two written exam. these test will consist of short questions relating concepts, definitions (30%) and a block multi-choice questions (15%).

1.2 Test at the end of each bloc. These proofs will consist of multi-choice questions. (15%)

2.Problems (25%)

2.1 Resolution of exercises in class (10%)

2.2 Problems written exam (15%)

3. Seminars (15% of module)

3.1 An individual written testwill be done with the content of the seminar sessions.(10%)

3.2 Seminar summary and Participation during seminar class (5%)

Assessment Activities

Title	Weighting	Hours	ECTS	Learning Outcomes
Resolution of exercises in class	10%	0	0	1, 6, 7, 3, 5, 2, 4
Seminar summary and Participation during seminar class	5%	0	0	1, 6, 7, 3, 5, 2, 4

Bibliography

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