

IDENTIFICATION DETAILS

Degree:	Biotechnology			
Scope	Biology and Genetics			
Faculty/School:	Experimental Sciences			
Course:	BASIC LABORATORY TECHNIQUES			
Туре:	Compulsory		ECTS credits:	6
Year:	2		Code:	2024
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Teaching period:	Third semester			
Subject:	Instrumental Techniques			
Module:	Experimental Methods in Biotechnology			
Teaching type:	Classroom-based			
Language:	Spanish			
Total number of student study hours:	150			

SUBJECT DESCRIPTION

This course focuses on the study of instrumental techniques that, due to their fundamental nature, are essential tools for biotechnologists. The program consists of three blocks. The first block studies the fundamentals and applications of techniques aimed at the isolation and characterization of biopolymers, chromatography, electrophoresis and centrifugation. The second covers basic techniques for the detection and quantification of biological compounds, radioisotopes, UV-visible absorption and fluorescence emission. The third block covers an internship in the laboratory for the experimental application of the techniques studied in blocks 1 and 2.

Biotechnology is an area of science with a great impact on daily life and its development is allowing great advances in many areas such as Biomedicine, forensic science, animal improvement, food products, development of genetically modified organisms for application in different fields, etc. As is the case in all scientific areas, knowledge advances are linked to technological development and the field of biotechnology is a clear reflection of this. Although the methods of Biotechnology are very diverse in nature, complexity and specificity, knowledge of certain basic instrumental techniques is essential for students of Biotechnology, not only because of their great application as tools for scientific research, because they are the theoretical and practical basis of other more advanced technologies.

This course consists of three blocks. The first block studies the physico-chemical fundamentals and applications of techniques aimed at the isolation and characterization of biopolymers: electrophoresis, centrifugation and chromatography. The second block covers mandatory techniques for the detection and quantification of biological compounds: UV-visible absorption, fluorescence emission and the use of radioactive isotopes. The third block covers practices in the laboratory in which, in addition to the knowledge of the equipment used for the development of the techniques studied, the ability to analyze results and obtain conclusions derived from experimental work will be acquired.

Knowledge of the fundamentals of the techniques on an individual basis must necessarily be completed with an understanding of their suitability for a given biological proposal. Hence the need to use the knowledge acquired in other subjects such as Cell Biology, Biochemistry or Genetics in order to successfully develop the proposed experimental proposals. This exercise forces the student to relate content and to think and work in an integrated way. That the student acquires the ability to propose and develop experimental proposals in the laboratory for the study of biomolecules and biological compounds using a single technique or a combination of the techniques learned will be the challenge of this program. The student will discover how methodological possibilities are fundamental tools and determine the advancement of scientific knowledge. Throughout the course, we work on the competence to analyze, interpret and obtain conclusions from experiments based on evidence and understanding the limitations of the scientific method as an applied research method.

GOAL

The general objective of this course is that the student, knowing the physico-chemical basis of the most commonly used instrumental techniques in a biotechnology laboratory, acquires the ability and skill to plan and adapt the use of the techniques studied to the separation, purification and detection of biomolecules and biological particles. The student will learn to integrate knowledge from the areas of Biology, Biochemistry, Genetics, Microbiology... in order, based on a deep understanding of the biological system being studied, to propose and develop an experimental proposal that answers the question posed.

The specific aims of the subject are:

That the student learns to properly design and execute an experimental protocol based on the theoretical knowledge of the various subjects||That the student learns to apply the theoretical knowledge acquired to the resolution of problems and practical cases related to the different subjects.

That the student develops criteria for problem solving in the context of a research protocol.

PRIOR KNOWLEDGE

Those corresponding to the Degree. Basic knowledge of Physics. Basic knowledge of Chemistry and Biochemistry: balance, acid-base, molecular atomic orbitals, structure of biomolecules. Basic knowledge of Biology.

COURSE SYLLABUS

TOPIC 1. Electrophoresis. Physico-chemical basis of the technique. Protein electrophoresis: Types. Features. Applications. Nucleic acid electrophoresis: Types. Features. Applications.

TOPIC 2. Centrifugation techniques. Physico-chemical basis of the technique. Types. Features. Applications.

TOPIC 3. Chromatography. Physico-chemical basis of the technique. Types. Features. Applications

TOPIC 4. UV-VIS absorption. Physico-chemical basis of the technique. Types. Features. Applications.

TOPIC 5. Fluorescence. Physico-chemical basis of the technique. Types. Features. Applications.

TOPIC 6. Isotopic Techniques. Physico-chemical basis of the technique. Types. Features. Applications.

TOPIC 7. Laboratory Practices

TOPIC 1. Electrophoretic techniques. Introduction and methodology. Types of electrophoresis: free and zonal. Basic equipment. Electrophoresis on paper. Applications. Protein electrophoresis. Characteristics and preparation of the support Non-denaturating electrophoresis. Denaturating electrophoresis. Two-dimensional electrophoresis. Detection methods. Densitometric quantification. Protein electrotransfer (Western blotting). Immunodetection. Nucleic acid electrophoresis. Characteristics and preparation of the support Non-denaturating electrophoresis. Denaturating electrophoresis. Electrophoresis in polyacrylamide gels. Electrotransfer of nucleic acids (Southern and Northern blotting). Pulsating field electrophoresis. Capillary electrophoresis. Characteristics and preparation of the support Electroendosmal flow. Applications.

TOPIC 2. Centrifugation Techniques. Subcellular fractionation techniques. Centrifugation theory. Centrifugation techniques. Differential centrifugation. Gradient centrifugation: zonal and isopyclic. Instrumentation: Centrifuges and rotors. Choice of centrifugation medium. Density gradient formation and analysis Practical applications. Subcellular fractionation. Determination of the sedimentation coefficient. Determination of complexes. DNA centrifugation.

TOPIC 3. Chromatography. Introduction. Classification. Chromatography on paper and thin layer: Description of the technique. Support. Application. Development. Detection. Column Chromatography: Description of the technique. Support. Application. Development. Detection. Spine efficiency. Band widening. Kinetic variables.
Optimization. Molecular exclusion chromatography: Description of the technique. Support. Application.
Development. Detection. Ion exchange chromatography: Description of the technique. Support. Application.
Development. Detection. Hydrophobic Chromatography: Description of the technique. Support. Application.
Development. Detection. Affinity Chromatography: Description of the technique. Support. Application.
Development. Detection. Chromatography: Description of the technique. Support. Application.
Development. Detection. Affinity Chromatography: Description of the technique. Support. Application.
Development. Detection. Chromatography on hydroxyapatite. High performance liquid chromatography: HPLC.
TOPIC 4. UV-visible absorption spectroscopy. Theoretical basis. Electronic transitions. Chromophore concept.
Lambert-Beer equation. Absorption spectrum. Equipment. Protein spectrophotometry. Nucleic acid
spectrophotometry. Colorimetry. Turbidometry. Kinetic measurements. Application to enzymatic assays.
Differential spectroscopy.

TOPIC 5. Fluorescence emission spectroscopy Theoretical basis. Emission spectra Excitation spectra. Spectrofluorimeters. Concentration measures. The phenomenon of fluorescence deactivation (Quenching). Energy transfer processes. Intrinsic and extrinsic fluorophores. Protein fluorescence. Nucleic acid fluorescence. Foundations of flow cytometry.

TOPIC 6. Isotopic techniques Introduction: Atomic nucleus. Elementary particles. Nuclear disintegrations. Stability of the atomic nucleus. Measurement of radioactivity. Accountants. Biological applications of isotopic techniques. Dissolution of radioactive compounds. Determination of unknown volumes. Determination of intracellular concentrations. Enzyme and transport assays. Radioactive tracers. Markings. Autoradiography. Beta image detectors.

TOPIC 7. Laboratory practices: Four laboratory practices will be carried out that will include the techniques of: PAGE-SDS Electrophoresis; Molecular Exclusion Chromatography, Absorption Spectroscopy, Gradient Centrifugation.

EDUCATION ACTIVITIES

- Participatory expository class: theoretical classes taught by the teacher for the study and consolidation of knowledge necessary for the understanding and correct implementation of the laboratory techniques essential for a biotechnologist.

- Practical classes: exercises, practical cases and/or experimental work carried out in the laboratory: throughout the semester, students will be asked to solve different tasks related to the contents studied (simulators, solving questions, carrying out tests, solving practical cases, analyzing articles or scientific news, etc...), individually or in groups. The cooperative learning methodology will be worked on. In addition, the experimental development of practical work will be carried out in the laboratory, applying techniques and knowledge related to the subject. In advance, the student will have the internship script and all the necessary independent study material for the proper use of their stay in the laboratory.

- Seminars, round tables, workshops, tutoring, debates, etc.: through tutoring (individual or group), the teacher, at his own request or the student and at the established time for this purpose, will answer questions or discuss the questions posed by the student, in order to guide him in learning the subject.

DISTRIBUTION OF WORK TIME

TEACHER-LED TRAINING ACTIVITIES	INDIVIDUAL WORK
64 Horas	86 Horas

SKILLS

Basic Skills

Students must have demonstrated knowledge and understanding in an area of study that is founded on general secondary education. Moreover, the area of study is typically at a level that includes certain aspects implying knowledge at the forefront of its field of study, albeit supported by advanced textbooks

Students must be able to apply their knowledge to their work or vocation in a professional manner and possess skills that can typically be demonstrated by coming up with and sustaining arguments and solving problems within their field of study.

Students must have the ability to gather and interpret relevant data (usually within their field of study) in order to make judgments that include reflections on pertinent social, scientific or ethical issues

Students must be able to convey information, ideas, problems and solutions to both an expert and non-expert audience

Students must have developed the learning skills needed to undertake further study with a high degree of independence

To have acquired the ability for analytical, synthetic, reflective, critical, theoretical and practical thought.

Capacity for problem-solving and decision-making.

To be able to plan time effectively.

To develop capacity for and a commitment to learning and personal development.

To develop oral and written communication skills.

To understand the fundamental laws and principles of physics, mathematics, chemistry and biology as the foundation for the mental structure of a biotechnician.

To acquire the skills needed for experimental work: design, preparation, the compilation of results and the obtainment of conclusions, understanding the limitations of an experimental approach.

General Skills

To have acquired the ability for analytical, synthetic, reflective, critical, theoretical and practical thought.

Capacity for problem-solving and decision-making.

To be able to plan time effectively.

To develop capacity for and a commitment to learning and personal development.

To develop oral and written communication skills.

To understand the fundamental laws and principles of physics, mathematics, chemistry and biology as the foundation for the mental structure of a biotechnician.

To acquire the skills needed for experimental work: design, preparation, the compilation of results and the obtainment of conclusions, understanding the limitations of an experimental approach.

Specific skills

Know how to properly design and execute an experimental protocol based on the theoretical knowledge of the various subjects.

Develop habits of rigorous thinking.

Know how to apply the theoretical knowledge acquired to solving problems and practical cases related to different subjects.

Develop criteria for problem solving and decision-making both in the professional and personal spheres.

Cultivate an attitude of intellectual concern and the search for truth in all areas of life.

LEARNING RESULTS

It extracts results from honestly carried out experiments

It understands the physicochemical basis of the basic instrumental techniques used in a biotechnology laboratory: chromatography, electrophoresis, centrifugation, UV-visible absorption, fluorescence and radioisotopes.

Solve exercises and practical cases related to the instrumental techniques studied.

It relates contents of other subjects such as Cell Biology, Biochemistry and Genetics in the application of the techniques studied to solve practical assumptions for the study and characterization of biomolecules and biological compounds.

Argues about the suitability of possible instrumental techniques for the separation, purification and determination of biomolecules and biological particles.

Learn about the management of the equipment used in the laboratory for the development of the instrumental techniques studied.

Correctly formulate a scientific hypothesis and propose appropriate experiments for verification.

Carry out laboratory experiments correctly applying the techniques studied.

Interpret correctly and applying scientific rigor the experimental results obtained in the laboratory.

Obtains and argues correctly and with scientific rigor conclusions based on the experimental results obtained in the laboratory.

LEARNING APPRAISAL SYSTEM

Participation in practical laboratory classes and group tutoring sessions will be a 'conditio sine qua non' for passing the subject. Plagiarism, as well as the use of illegitimate means in evaluation tests, will be sanctioned in accordance with those established in the Evaluation Regulations and the University's Coexistence Regulations. ORDINARY EVALUATION SYSTEM (this is the default evaluation system for students in this subject) - Evaluation of the experimental work carried out in university laboratories: 30%. The ability to solve experimental problems, the interpretation of research results and the written presentation of these results will be evaluated. A

minimum grade of 5 will be required in this section to pass the subject.

- Evaluation of the theoretical content of the subject: 60%. The exam will consist of short questions and problems. A minimum score of 4.5 on the exam will be required to pass the subject.

- Evaluation of seminars: carrying out and presenting exercises, case studies, debates, tutorials, etc.: 10%. At the end of the first block, a test consisting of exercises and practical cases will be carried out. Matter will be eliminated if the grade is equal to or greater than 7.

ALTERNATIVE EVALUATION SYSTEM (only in the case of students in the second call and later, and students with academic exemption, who request it in a reasoned manner by mail from the teachers of the subject during the first week of class). If you do not report, the ordinary evaluation system will be assumed.) In the alternative evaluation system, the following percentages will be applied:

- Evaluation of seminars: carrying out and presenting exercises, case studies, debates, tutorials, etc.: 30%. The qualification obtained in internships and the resolution of practical cases will be maintained until the extraordinary call of the current academic year.

- Evaluation of the theoretical content of the subject: 60%. The exam will consist of short questions and problems. A minimum score of 4.5 on the exam will be required to pass the subject.

- Evaluation of seminars: carrying out and presenting exercises, case studies, debates, tutorials, etc.: 10%. At the end of the first block, a test consisting of exercises and practical cases will be carried out. Matter will be eliminated if the grade is equal to or greater than 7.

Plagiarism, as well as the use of illegitimate means in evaluation tests, will be sanctioned in accordance with those established in the Evaluation Regulations and the University's Coexistence Regulations.

ETHICAL AND RESPONSIBLE USE OF ARTIFICIAL INTELLIGENCE

1.- The use of any Artificial Intelligence (AI) system or service shall be determined by the lecturer, and may only be used in the manner and under the conditions indicated by them. In all cases, its use must comply with the following principles:

a) The use of AI systems or services must be accompanied by critical reflection on the part of the student regarding their impact and/or limitations in the development of the assigned task or project.

b) The selection of AI systems or services must be justified, explaining their advantages over other tools or methods of obtaining information. The chosen model and the version of AI used must be described in as much detail as possible.

c) The student must appropriately cite the use of AI systems or services, specifying the parts of the work where they were used and describing the creative process followed. The use of citation formats and usage examples may be consulted on the Library website(<u>https://www.ufv.es/gestion-de-la-informacion_biblioteca/</u>).

d) The results obtained through AI systems or services must always be verified. As the author, the student is responsible for their work and for the legitimacy of the sources used.

2.- In all cases, the use of AI systems or services must always respect the principles of responsible and ethical use upheld by the university, as outlined in the <u>Guide for the Responsible Use of Artificial Intelligence in Studies at UFV</u>. Additionally, the lecturer may request other types of individual commitments from the student when deemed necessary.

3.- Without prejudice to the above, in cases of doubt regarding the ethical and responsible use of any AI system or service, the lecturer may require an oral presentation of any assignment or partial submission. This oral evaluation shall take precedence over any other form of assessment outlined in the Teaching Guide. In this oral defense, the student must demonstrate knowledge of the subject, justify their decisions, and explain the development of their work.

BIBLIOGRAPHY AND OTHER RESOURCES

Juan Manuel Garcia-Segura... [et al.]. Instrumental analysis techniques in biochemistry/Madrid: Synthesis, 2008.

D. Freifelder. Molecular biology and biochemistry: problems and applications/W.H. Freeman.

Additional

Joseph Sambrook, Michael R. Green. Molecular cloning: a laboratory manual/4th ed. New York: Cold Spring Harbor Laboratory Press, 2014.