COURSE DATA

DATA SUBJECT

Code: 36882
Name: Methods in Biochemistry and Molecular Biology II
Cycle: Undergraduate Studies
ECTS Credits: 7.5
Academic year: 2025-26

STUDY (S)

Degree	Center	Acad. year	Period
1111 - Grado en Biotecnología	Facultat de Ciències Biològiques	2	Second quarter

SUBJECT-MATTER

Degree	Subject-matter	Character
1111 - Grado en Biotecnología	Biochemistry methodology	COMPULSORY

COORDINATION

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SUMMARY

The development of separative methods for the isolation and purification as well as for the characterisation of biomolecules in Biochemistry and Molecular Biology has had, and will continue to have, a great impact on the development of Biotechnology. This subject responds to the need to develop specific tools and skills in an experimental scientific discipline such as Biotechnology. Together with Methods in Biochemistry and Molecular Biology I, the course belongs to the subject Biochemical Methodology and both subjects introduce students to the fundamentals and applications of the basic methodologies of analysis, characterisation and separation of biomolecules and their different groupings or associations. In particular, the programme of Methods in Biochemistry and Molecular Biology II has been designed for the second year of the degree in Biotechnology, after the teaching of Methods I in the first four-month period of the same year, and is not a definitive proposal, as the emergence of new techniques or the updating of existing ones suggests that they should be incorporated into the programme. Each topic covers a technique or set of related techniques for the separation, purification and isolation of biomolecules and/or associations of biomolecular components. The development of the content of the topics includes an introduction to the fundamentals of the method or set of procedures, as well as discussion of their experimental use and possible applications. Multiple examples of applications will be described.

PREVIOUS KNOWLEDGE

RELATIONSHIP TO OTHER SUBJECTS OF THE SAME DEGREE

There are no specified enrollment restrictions with other subjects of the curriculum.

OTHER REQUIREMENTS

COMPETENCES / LEARNING OUTCOMES

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Actuar con autonomía en el aprendizaje, tomando decisiones fundamentadas en diferentes contextos, emitiendo juicios en base a la experimentación y el análisis y transfiriendo el conocimiento a nuevas situaciones

Adquirir conocimientos de los fundamentos físico-químicos y las bases metodológicas de las técnicas utilizadas en estudios moleculares

Apply analytical, synthetic and critical thinking skills in the application of the scientific method.

Colaborar eficazmente en equipos de trabajo, asumiendo responsabilidades y funciones de liderazgo y contribuyendo a la mejora y desarrollo colectivo

Comprender protocolos de separación, caracterización y análisis de moléculas biológicas

Contribuir en el diseño, desarrollo y ejecución de soluciones que den respuesta a demandas sociales, teniendo en cuenta como referente los Objetivos de Desarrollo Sostenible

Demostrar razonamiento crítico y autocrítico en el ámbito de la titulación, considerando aspectos tales como la ética profesional, los valores morales y las implicaciones sociales de las diferentes actividades realizadas

Design and carry out a complete protocol for obtaining and purifying a biotechnological product.

Diseñar protocolos de separación, purificación y caracterización de moléculas biológicas

Manejar adecuadamente los equipos y el material propio de un laboratorio de bioquímica y biología molecular

Participate in multidisciplinary teams, engaging in teamwork and collaboration.

Propose creative and innovative solutions to complex situations or problems, typical of the area of ¿¿¿¿connection, to donate responses to the various professional and social needs

Saber comunicarse de manera efectiva, tanto de forma oral como escrita, adaptándose a las características de la situación y de la audiencia

Use English to write reports and to interpret information from protocols, manuals and databases.

Work in laboratories, including safety procedures, waste management and accurate activity logging.

DESCRIPTION OF CONTENTS

Topic 1: Properties and characterization of biomolecules

The study of vital phenomena. In vivo and in vitro experiments. Extraction and purification of biological molecules. Disorganization of living matter. Total and limited homogenization. Isolation and purification of macromolecules. Preliminary separations. Precipitation and dialysis. Preparative and analytical separation methods. Resolution, yield and degree of purification.

Topic 2. Centrifugation

Introduction: Fundamentals and definitions. Instrumentation: Centrifuges and rotors. Centrifugation modes: Differential centrifugation. Zonal centrifugation. Density gradient centrifugation, procedures. Isopycnic centrifugation. Centrifugation applications Special preparation rotors Analytical ultracentrifugation: Ultracentrifuges, rotors and analytical centrifuge cells. Applications: Determination of sedimentation coefficients and molecular masses.

Topic 3. Chromatography

Introduction: definitions, generalities, nomenclature. Basis and classification of chromatographic methods. Chromatography on flat surfaces: paper chromatography and thin layer chromatography. Column chromatography: fundamentals and basic operations. Chromatographic parameters. Capacity and resolution: selectivity and efficiency, theoretical plate concept. Adsorption chromatography: Ion exchange chromatography, types and forms of elution, chromatofocusing; Hydrophobicity chromatography; Affinity chromatography, preparation of the stationary phase, elution modes; Artificial affinity chromatography, chromatography on immobilised metals-IMAC, chromatography on immobilised dyes; Other types of adsorption chromatography, chromatography on hydroxyapatite. Molecular exclusion chromatography, rationale and applications. High performance liquid chromatography-HPLC: instrumentation; stationary phases, column types; reverse phase HPLC-RP. Chromatography on magnetic matrices.

Topic 4. Electrophoresis

Introduction: Basis and definitions. Staining methods. Free and zonal electrophoresis. Capillary electrophoresis. Electrophoresis on non-restrictive supports. Applications.

Protein electrophoresis on restrictive supports: Ferguson representation, Polyacrylamide gel electrophoresis (PAGE), Electrophoresis in discontinuous system, Electrophoresis under denaturing conditions (SDS-PAGE), Polyacrylamide Gradient Gel Electrophoresis (PAGGE). Electrophoretic transfer and immunodetection (Western-blot). Isoelectrofocusing and two-dimensional electrophoresis.

Applications.

Nucleic acid electrophoresis. Agarose gels. Transfer to membranes. Applications.

Topic 5. Mass Spectroscopy of Biomolecules

Introduction. The mass spectrometer: ionization modes; mass analyzers; fragmentation methods. Mass spectra: intensity; mass/charge ratio; resolution; isotopic variability and calculation of the mass of an ion. Biochemical applications: Proteomics and Metabolomics: identification, quantification and acquisition methods.

6. Laboratory practices

A practical to be developed in 5 sessions of 4 hours, during the same week (intensive practice)

Practice: Purification and analysis of ribulose-1,5-bisphosphate carboxylase oxygenase (RubisCO) from orange tree leaves. We will start from plant tissue and after the extraction of total proteins we will initiate successive steps of RubisCO purification using differential precipitation, centrifugation, dialysis and ion exchange chromatography. Aliquots will be taken throughout the purification process for subsequent analysis by spectrophotometric quantification of total proteins and denaturing polyacrylamide gel electrophoresis with SDS (SDS-PAGE). Finally, image analysis of the polyacrylamide gel will be performed to calculate the yield and purification factor throughout the purification process. Likewise, the molecular mass of the two subunits that compose the enzyme will be analyzed and their stoichiometric ratio will be estimated.

WORKLOAD

PRESENCIAL ACTIVITIES

Activity	Hours
Theory	40,00
Laboratory	20,00
Classroom practices	15,00
Total hours	**75,00**

NON PRESENCIAL ACTIVITIES

Activity	Hours
Attendance at other activities	0,00
Individual or group project	0,00
Independent study and work	40,00
Preparation of lessons	55,00
Preparation for assessment activities	17,50
Resolution of case studies	0,00
Total hours	**112,50**

TEACHING METHODOLOGY

Theory classes: The course is designed to promote active learning of students. Thus, the theoretical classes are conceived as introductions to each topic, presenting the most relevant contents using mainly audiovisual media, and where research techniques will be described and an attempt will be made to give a global and interrelated vision of them.

Prior to the theoretical classes, students will have bibliographic information and material provided by the professor and published in the Virtual Classroom. It is intended that in these classes there will be an active participation of the students. This participation will be encouraged through the resolution of questions. The teacher may request the delivery of solved questions. This activity will allow to know how the students assimilate the concepts and thus to better evaluate the student's work.

Classes of problems. The classes of problems will be posed in such a way that the students solve part of them in the classroom under the supervision of the teacher and sometimes working in teams with other classmates. The students will have from the beginning of the course lists of proposed problems related to the different topics of the program. The discussion of scientific articles may also be carried out, where students, together with the teacher, will comment on a research article, proposed by the teacher, related to the topics of the program. Afterwards, a series of questions about the objectives, methodology, results and conclusions of the research work will be solved.

Practical laboratory sessions: Prior to the practical classes, students will have bibliographic information and material available through the Virtual Classroom. The professor will provide the student with a booklet/guide that will contain not only the protocols to follow but also bibliographical references and some self-evaluation questions to be solved during the practical sessions. This will ensure that the students have the basic knowledge to carry out and make the most of the practical tasks.

The practical sessions will be planned in such a way that the students will participate in the performance of the experiments, including the development of the practice, the collection of data and the process of elaboration and interpretation of the results to finally provide conclusions of the experiment. All this in the teaching laboratory and under the supervision of the teacher and working as a team with colleagues. At the end of the practices, students will solve and present a ¿results questionnaire¿, with the results obtained and the conclusions drawn, to also demonstrate their ability to formalize and communicate scientific data.

EVALUATION

There will be a written test on the contents (theory, problems and practices) of the program of the course that will determine 80% of the grade of the course. This exam will have to be passed (grade of 5 out of 10) in order to pass the course.

The evaluation of the laboratory practices will represent 15% of the final grade of the course and will be obtained through the individualized follow-up of the student's activities, through the elaboration of questionnaires and his active participation in class. Attendance to all the laboratory sessions is

indispensable for the evaluation of this part of the course.

The final grade of the course will take into account the student's participation in the classes (5% of the grade).

An overall grade equal or higher than 5 out of 10 will be necessary to pass the course, having passed each of the parts: written tests and laboratory practices. The laboratory and class participation grades will be added once the written exams have been passed.

Evaluation of the second call. It will follow the same evaluation criteria that have been exposed for the first call and will consist of a single exam on all the contents of the subject (80%), incorporating the qualification of the laboratory practices (15%) and class participation (5%).

In case the laboratory practices are approved, but not the subject as a whole, the qualification of practices will be maintained for the following course.

REFERENCES

Basic references

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Complementary references

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