# COURSE DATA

| Data Subject | |
|---|---|
| **Code** | 33138 |
| **Name** | Methods in biochemistry |
| **Cycle** | Grade |
| **ECTS Credits** | 12.0 |
| **Academic year** | 2022 - 2023 |

**Study (s)**

| Degree | Center | Acad. year | Period |
|---|---|---|---|
| 1109 - Degree in Biochemistry and Biomedical Sciences | Faculty of Biological Sciences | 2 | Annual |

**Subject-matter**

| Degree | Subject-matter | Character |
|---|---|---|
| 1109 - Degree in Biochemistry and Biomedical Sciences | 10 - Métodos instrumentales | Obligatory |

# SUMMARY

The course of "Methods in Biochemistry" is included within the subject "Methods in Molecular Biosciences", which is compulsory in the second degree course in Biochemistry and Biomedical Sciences. This course has 27 ECTS credits and it is offered through annual length in four courses: "Methods in Biochemistry" (12 ECTS), "Genetic Engineering" (6 ECTS), "Techniques of genetic analysis" (4, 5 ECTS credits) and "Cell analysis techniques" (4.5 ECTS).

The general objectives of the course "Methods in Biochemistry" are: 1) Describe the fundamentals of the methods in the field of Biochemistry and Molecular Biology. 2) Be familiar with techniques to purify, characterize and manipulate biomacromolecules. 3) Analyze the application of the studied methodologies to solve biological problems.

# PREVIOUS KNOWLEDGE

## Relationship to other subjects of the same degree

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

## Other requirements

# OUTCOMES

## 1109 - Degree in Biochemistry and Biomedical Sciences

- Have capacity for analysis, synthesis and critical reasoning in the application of the scientific method.
- Be able to think in an integrated manner and approach problems from different perspectives.
- Be able to use new information and communication technologies.
- Know how to use the different bibliographic sources and biological databases and be able to use bioinformatic tools.
- Know the usual procedures used by scientists in the area of molecular biosciences and biomedicine to generate, transmit and disseminate scientific information.
- Understand experimental approaches and their limitations and interpret scientific results in molecular biosciences and biomedicine.
- Know how to design multidisciplinary experimental strategies in the field of molecular biosciences to solve complex biological problems, especially those related to human health.
- Acquire skills to use the methodologies of molecular biosciences and to keep an annotated record of activities.
- Know how to work responsibly and rigorously in the laboratory, considering the safety aspects in experimentation as well as the legal and practical aspects of the handling and disposal of waste.
- Students must have acquired knowledge and understanding in a specific field of study, on the basis of general secondary education and at a level that includes mainly knowledge drawn from advanced textbooks, but also some cutting-edge knowledge in their field of study.
- Students must be able to apply their knowledge to their work or vocation in a professional manner and have acquired the competences required for the preparation and defence of arguments and for problem solving in their field of study.
- Students must have the ability to gather and interpret relevant data (usually in their field of study) to make judgements that take relevant social, scientific or ethical issues into consideration.
- Students must be able to communicate information, ideas, problems and solutions to both expert and lay audiences.
- Students must have developed the learning skills needed to undertake further study with a high degree of autonomy.

- Show initiative and leadership for multidisciplinary teamwork and cooperation.
- Be able to assimilate scientific texts in English.

## LEARNING OUTCOMES

*In theoretical and problem classes:* Acquire knowledge of the physicochemical bases and methodological techniques used in molecular studies.

- Acquire knowledge of the main analytical and separative techniques used in field of Biochemistry and Molecular Biology.
- Understand and evaluate the methodology used in scientific work related to Biochemistry and Molecular Biology.
- To be able to design protocols for separation, purification and characterization of biological molecules.
- To be able to perform calculations to obtain quantitative information on biological molecules and processes from data provided by analytical techniques.

*In practical sessions:*

- To know how to properly handle the equipment and the basic material present in a laboratory of Biochemistry.
- To understand and be able to follow correctly protocols for separation, characterization and analysis of biological molecules.
- To be able to discuss the experimental results and correctly prepare a technical report from them.

## DESCRIPTION OF CONTENTS

### 1. ITEM 1. ABSORPTION SPECTROSCOPY.

Physicochemical fundamentals of spectroscopy. Measure the absorption of radiation. Molecular absorption spectroscopy in the ultraviolet-visible (UV-V). Spectroscopy in the region of infrared (IR). Biochemical applications. (12 hours T, 3 hours P)

## 2. ITEM 2. FLUORESCENCE SPECTROSCOPY.

Dissipation of energy by excited molecules. Fluorescence and chemiluminescence. Fluorescence spectroscopy, generalities. Induced energy transfer by resonance. Biochemical applications: intrinsic and extrinsic fluorescence of proteins, nucleic acids and membranes. Cellular studies. (9 hours T, 3 hours P)

## 3. ITEM 3. NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY.

Basis of MRI. Couplings and NOE. Multidimensional NMR. Applications: in vitro studies, in vivo studies. (5 hours T, 1 hour P)

## 4. ITEM 4. CHROMATOGRAPHY.

General fundamentals of chromatography. Partition chromatography. Adsorption chromatography. Ion exchange chromatography. Exclusion chromatography. Affinity chromatography. Biochemical applications. (6 hours T, 3 hours P)

## 5. ITEM 5. ELECTROPHORESIS.

General. Electrophoresis techniques. Free electrophoresis capillary. Zonal electrophoresis in polyacrylamide and agarose. Transfers to other media. Applications. Isoelectric focusing. Two-dimensional electrophoresis. (5 hours T, 2 hours P)

## 6. ITEM 6. CENTRIFUGATION.

The process of sedimentation. Sedimentation coefficient. Preparative sedimentation homogeneous medium. Sub cellular fractions in density gradient sedimentation, and isopycnic zonal. (2 hours T, 2 hours P)

## 7. ITEM 7. ISOTOPIC METHODS.

Fundamental principles of the use of isotopes in biochemistry. Radioactive decay. Measurement of radioactivity. Applications: in vivo and in vitro. Radioimmunoassays. Autoradiography: autoradiographic methods. (6 hours T, 3 hours P)

## 8. ITEM 8. MASS SPECTTROMETRY.

Introduction. Mass spectra and load / mass. Mass spectrometry of high molecular weight compounds. Biochemical applications. (5 hours T, 2 hours P)

### 9. Practical sessions of Llaboratory

PRACTICE 1. INTRODUCTION TO THE ABSORPTION SPECTROSCOPY. Management of the spectrophotometer. The Lambert-Beer Law: determination of the extinction coefficient of hemoglobin. Influence of pH on the absorption spectrum. Analysis of mixtures of chromophores. (4h).

PRACTICE 2. STUDY OF ENZYMATIC KINETIC BY SPECTROSCOPY. Measurement of alkaline phosphatase activity by spectrophotometry. Determination of kinetic parameters and types of inhibición. Hyperchromic effect. Determination of nuclease activity by hyperchromic effect. Calculation of hyperchromicity of DNA (4h).

PRACTICE 3. MEASUREMENT OF ENZYME ACTIVITY AND METABOLITES BY SPECTROSCOPY. Determination of nuclease activity by hyperchromic effect. Calculation of a DNA hyperchromicity. Determination of ethanol concentration in samples by enzymatic assay. (4h)

PRACTICE 4. INTRODUCTION TO FLUORESCENCE SPECTROSCOPY. STUDY OF PROTEIN-LIGAND INTERACTION. Management of the spectrofluorimeter. Excitation and emission spectra. Influence of solvent polarity on the emission spectrum of a fluorophore. Fluorescence protein. Study of protein-ligand interaction. (4h).

PRACTICE 5. DETERMINATION OF Ca2+ CONCENTRATION AND pH USING FLUORESCENT PROBES. Determination of intracellular calcium and intracellular pH using permeable fluorophores. (4)

PRACTICE 6. SEPARATIVE TECHNIQUES. Purification of a protein (RUBISCO) using differential precipitation, dialysis, chromatography and polyacrylamide gel electrophoresis. Yield analysis and purification factor. (20h)

## WORKLOAD

| ACTIVITY | Hours | % To be attended |
|---|---|---|
| Theory classes | 61,00 | 100 |
| Laboratory practices | 40,00 | 100 |
| Classroom practices | 19,00 | 100 |
| Attendance at events and external activities | 2,00 | 0 |
| Development of group work | 15,00 | 0 |
| Study and independent work | 98,00 | 0 |
| Preparation of evaluation activities | 14,00 | 0 |
| Preparing lectures | 25,00 | 0 |
| Preparation of practical classes and problem | 20,00 | 0 |
| Resolution of case studies | 6,00 | 0 |
| **TOTAL** | **300,00** | |

## TEACHING METHODOLOGY

**Lectures of Theory:** A total of 50 sessions of an hour will be needed to cover this facet of teaching. This course aims to promote active learning by students. The lectures are intended as general introductions to each topic which will present the different research techniques and will try to give a global and co-related them. Prior to the lectures, the students will have bibliographic information and material provided by the teacher.

**Lectures of problems, questions and commentary of papers**: will be held 19 sessions of one hour during the entire course, interspersed with the lectures. They will be proposed to the students to solve some of the problems in the classroom under the teacher's supervision and promote teamwork with colleagues. There will also have comment of some scientific articles. The students will participate in a discussion led by the teacher about a research paper, provided by the teacher, which is related to the topic as well as series of questions about the article.

**Practical laboratory sessions**: The teacher will provide in advance to the student with a booklet containing not only the protocols to follow, but also references and some theoretical questions that students must solve (with the help of the literature) before practice. In the first session of each practice, the teacher will discuss these issues with the students ensuring they have adequate knowledge to perform the practical use. Once the experiments are done, the students must submit a technical report containing the results and conclusions that can be drawn from them. The attendance at the practical sessions is mandatory.

## EVALUATION

The assessment of learning will be completed according to the following criteria:

a) 80% of the mark will come from written tests where it will be considered the student knowledge and its ability to apply this knowledge to the interpretation of experimental results and to the resolution of questions and problems related to the experimental methodology employed. The exams will consist of one part of theoretical questions and problems.

b) 20% of the mark will come from the evaluation of practical sessions. This mark will valuate how students developed the practice (provided solutions to theoretical issues outlined above), conducted the experiments (degree of understanding and care in following the protocols) and the final technical report (presentation, clarity and appropriateness of the conclusions obtained).

There will be two qualifying exams. The first will valuate the knowledge for the analytical techniques group of items (items 1 to 3). The second examination shall cover the topics of radioactivity and separation techniques (items 4 to 8). Each exam is scored on 10 points. The average of the two tests are multiplied by a factor 0.8, to be added the qualification of practical sessions (a total of 2 points) for the

overall rating of the subject. The earning of a mark below 4.0 in any of the exams, an average value of the two exams below 4.5, or an overall course mark below 5.0, will force to a re-evaluation in a second annual convocation. The mark of one of the parts can be keep up to the second convocation just if the score is above 4.5.

Exceptionally, the final mark can be enhanced by an outstanding student participation in resolution of the proposed questions solution and in the discussion of research articles that demonstrate, in the opinion of the teacher, an extraordinary level of knowledge. In any case, the contribution of the latter section will not exceed 1 point in the final mark.

To pass of the course is mandatory to do the practical sessions.

## REFERENCES

### Basic

- García Segura, J.M., Gavilanes, J.G., Martínez del Pozo, A., Montero, F., Oñaderra, M. Y Vivanco, F. Técnicas instrumentales de análisis en Bioquímica. Ed. Síntesis, 1996.
- Barceló Mairata, F. Técnicas instrumentales en Bioquímica y Biología. Ed. Universitat de les Illes Balears, 2003.
- Roca, P., Oliver, J. Y Rodriguez, A.M. Bioquímica. Técnicas y Métodos. Ed Hélice. 2004
- Freifelder, D. "Técnicas de bioquímica y biología molecular" Ed. Reverté, 1991

### Additional

- Sheehan, D. Physical biochemistry: Principles and Applications (2ª edición). Ed. Wiley-Blackwell, 2009.
- Wilson, K. y Walker, J. (eds.) "Principles and Techniques of Biochemistry and Molecular Biology" (6ª edición). Cambridge University Press, 2005
- Holme, D.J. y Peck, H. Analytical Biochemistry (3ª edición). Ed. Pearson Education Limited, 1998.
- Serdyuk, I.N., Zaccai, N. Zaccai, J. Methods in molecular biophysics Ed. Cambridge University Press, 2007.