COURSE DATA

Data Subject	
Code	33177
Name	Integrated internship methods
Cycle	Grade
ECTS Credits	4.5
Academic year	2023 - 2024

Study (s)

Degree	Center	Acad. year	Period
1102 - Degree in Biotechnology	Faculty of Biological Sciences	2	Second term

Subject-matter

Degree	Subject-matter	Character
1102 - Degree in Biotechnology	85 - Biochemistry methodology	Obligatory

Coordination

Name	Department
MARIN NAVARRO, JULIA VICTORIA	30 - Biochemistry and Molecular Biology
PEÑARRUBIA BLASCO, DOLORES	30 - Biochemistry and Molecular Biology

SUMMARY

The development of analytical methods in biochemistry and molecular biology has had and will continue to have a large impact on the development of biotechnology. This course is intended for students to know, contact and become familiar with most common experimental techniques of biochemistry and molecular biology. It will also try the student to develop specific practical skills indispensable in empirical scientific discipline. The course introduces students in managing basic instrumental in biochemistry and molecular biology laboratory, in obtaining physical and chemical parameters of biomolecules and in the interpretation of the resulting data. The program aims to complement the knowledge about the techniques and methodology used in chemistry laboratories, explained in the theoretical course (Methods in Molecular Biology and Biochemistry). A series of experiments related to the thematic order of most of the lessons of the theoretical program is proposed. In the Integrated Practices in Methods course, which is exclusively practical, the program is not static since the emergence of new techniques, methods and experimental procedures can advise the incorporation of these innovations to the program. Practical classes consist of 11 sessions with a total of 42 hours to be performed in two blocks (21 hours each). The first of these, consisting of 6 sessions to perform in different weeks (non-intensive) includes experiments spectrophotometry, spectrofluorometry and diverse applications. The experiences made in this first block correspond to the theoretical contents explained in the first term in the theoretical subject, Methods in

Biochemistry and Molecular Biology. The second block (5 sessions) to perform intensively in a week, includes experiments in separation and purification techniques, whose theoretical basis is explained in the second term in the theoretical subject, Methods in Biochemistry and Molecular Biology. The practical sessions will include a brief introduction to the fundamentals of method or group of methods used, experimental handling and how to process the data. Each practice may include a technique or a group of related techniques and various applications. The experiments to be performed are simple, easily performed so that they are pedagogical and can be interpreted by students after processing results. Attendance at all laboratory sessions is mandatory and necessary for the subject to be evaluated.

PREVIOUS KNOWLEDGE

Relationship to other subjects of the same degree

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

Other requirements

To take this course you need to have completed or be enrolled in the course on Methods in Biochemistry and Molecular Biology

OUTCOMES

1102 - Degree in Biotechnology

- Design protocols for the separation, purification and characterisation of biological molecules.
- Properly handle the equipment and material of a biochemistry and molecular biology laboratory.

LEARNING OUTCOMES

- Properly handle the equipment and the basic material of a laboratory of biochemistry and molecular biology.
- Understand and follow proper protocols for separation, characterization and analysis of biological molecules.
- Interpret and discuss the experimental results and prepare a technical report correctly on them.
- Ability to prepare, design, implement, interpret and discuss experiments in teams with other students.
- Ability to communicate with other students in the discussion of the methodology used in conducting the experiments.

DESCRIPTION OF CONTENTS

1. Part 1. Spectroscopic methods: absorption and fluorescence spectroscopy.

Implementation of four practices to be developed in two sessions of 4.5 hours, two sessions of 4 hours and two sessions of 2 hours, in different weeks (not intensive)

Practice 1: Introduction to UV-visible spectrophotometry. Colorimetry and spectrophotometry. Absorbance measurements of chromophores in solution. Calculation of extinction coefficient. Lambert-Beer law. Limitations of the law. Calculation of concentrations of solutes in mixtures. Study of hiperchromic effect.

Practice 2: Spectrophotometric quantification of metabolites and turbidometric analysis of enzymatic kinetics. Enzymatic and spectrophotometric determination of ethanol concentration. Determination of lipase activity.

Practice 3: Protein-ligand interaction followed by spectrofluorometry. Management of spectrofluorometer. Excitation and emission spectra. Using ANS as polarity sensor fluorophore. Analysis of protein-ligand interaction.

Practice 4: Fluorescent probes: spectrofluorometric determination of Ca2+ and pH in solutions. Analysis of the variation of the excitation spectra of fluorophores QUIN2 and 5'-carboxy-4',5'-dimethyl fluorescein with Ca2+ concentration and pH, respectively. Determination of Ca2+ and pH in problem solutions.

2. Part 2. Methods of separation and purification of biomolecules.

A practice to develop over five sessions (4 to 4.5 hours and 1 in 3 hours, intensive 1 week), using chromatographic methods, electrophoretic and basic centrifuge. Application to the purification, characterization and analysis of Rubisco protein.

Practice 5: Study of the ribulose-1 ,5-bisphosphate carboxylase oxygenase orange leaves. Separation techniques. Extraction and purification of a protein (RuBisCO) using differential precipitation, centrifugation, dialysis, chromatography and polyacrylamide gel electrophoresis. Performance analysis and purification factor.

WORKLOAD

ACTIVITY	Hours	% To be attended
Laboratory practices	42,00	100
Classroom practices	3,00	100
Study and independent work	12,00	0
Preparation of evaluation activities	18,00	0
Preparation of practical classes and problem	15,00	0
TOTAL	**90,00**	

TEACHING METHODOLOGY

Prior to the practical classes students will have bibliographic information and material through the Virtual Classroom. The teacher will provide the student a booklet / guide that will contain not only the protocols to follow but also references and a few self-evaluation questions to be solved along practical sessions.

The practical sessions will be raised so that the students participate in the experimental work, including practice development, obtaining data and the preparation and interpretation of the results to finally provide a conclusion of the experiment. All this in the laboratory and under the supervision of the teacher and working together with peers. At the end of practice (after 15 days for each part), students will submit a results questionnaire with the results and conclusions drawn, in order to demonstrate their ability to formalize and communicate scientific data.

EVALUATION

Part I (Practices 1-4) and Part II (Practice 5): a written test (examination) on the contents and activities during the practice sessions of the two parts of the course will be held. Each part will be worth 40% of the final grade. You must obtain a score of at least 1.8 / 4 (or 4.5 / 10) on each of the parts and a 5 in total to pass the course. Compensable scores will be saved only during the academic year. The remaining 20% of the grade will come from the assessment of student participation and responses to the issues raised during and after the completion of the practices, by evaluating the responses to the questionnaires submitted at the end of practice sessions of the two parts.

In the case of having failed the exam, the mark of the questionnaire will be kept until the following academic year and attendance at practices will be voluntary. To opt for a new qualification of the results questionnaire, it will be necessary to attend all the practical sessions.

REFERENCES

Basic

- Primera parte (prácticas 1-4)

-Bergmeyer, U. (1984) "Methods in enzymatic analysis" 3rd ed. Verlag Chemie
-Cornell, N.W. y Veech, R. (1983) "Enzymatic measurement of ethanol or NAD in acid extracts of biological samples". Anal. Biochem., 132, 418-423
-Cuatrecasas, P., Fuchs, S. y Anfinsen, C.B. (1967) "Catalytic properties and specificity of the extracellular nuclease of Staphylococcus aureus". J. Biol. Chem. 242, 1541-1547.
-Díaz, P,. y Daban, J.-R. (1986) "Enzymatic probes for histone-DNA complexes: micrococcal nuclease activity under conditions useful for the investigation of chromatin structure". J. Biochem. Biophys. Meth., 13, 57-59.
-Instructions for the analysis using test-combinations de Boehringer Mannheim Biochímica (1995) "Methods of enzymatic bioanalysis and food analysis". Boehringer Mannheim Biochemicals
-Moller, M. y Denicola, A. (2002) Study of protein-ligand binding by fluorescence Biochem. Mol. Biol. Edu. 30, 309-312.
-Sugihara, A. et al. (1986) Biochemistry 25, 3430
-Stryer, L. (1968) "Fluorescence spectroscopy of proteins" Science, 162, 526-533
-von Tigersrom, R.G. y Stelmaschuk, S. (1989) The use of Tween 20 in a sensitive turbidometric assay of lipolytic enzymes Can. J. Microbiol. 35, 512-514.
-Walker, J.R.L. (1992) "Spectrophotometric determination of enzyme activity: alcohol dehydrogenase (ADH)". Biochem. Educ., 20, 42-43.

- Segunda parte (práctica 5)

Andersson, I. (2008): Catalysis and regulation in Rubisco, J. Exp. Bot., 59, 1555-1568 (hemeroteca de ciencias).
Andersson, I. y Backlund, A. (2008): Structure and function of Rubisco. Plant Physiol. Biochem. 46, 275-291 (hemeroteca de ciencias).
Atha, D. H. y Inghamg, K.C. (1981): Mechanism of precipitation of proteins by polyethylene glycols. J. Biol. Chem., 256, 12108-12117 (hemeroteca de ciencias).
Deutscher, M.P. (ed.) (1990): Guide to protein purification, Academic press, San Diego (biblioteca del departamento).
Gutteridge, S. y Gatenby, A. A. (1995): RuBisCO synthesis, assembly, mechanism and regulation. Plant Cell 7, 809-819 (hemeroteca de ciencias).
Peñarrubia, L., Moreno, J. y Carrasco, P. (1988): A visual-electrophoretic method for following the purification of ribulose-1,5-bisphosphate carboxylase oxygenase. Biochem. Educ., 16, 234-236 (hemeroteca de ciencias).
Peñarrubia, L. y Moreno, J. (1988): Ribulose 1,5-bisphosphate carboxylase/oxygenase from citrus leaves. Phytochemistry, 27, 1999-2005 (hemeroteca de ciencias).
Schneider, G., Lindqvist, Y. y Brändén, C.I. (1992): RuBisCO: structure and mechanism. Annu. Rev. Biophys. Biomol. Struct., 21, 119-143 (hemeroteca de ciencias).
Spreitzer, R.J. y Salvucci, M.E. (2002): Rubisco: structure, regulatory interactions, and possibilities for a better enzyme. Annu. Rev. Plant Biol. 53, 449-475 (hemeroteca de ciencias).