# COURSE DATA

| Data Subject | |
|---|---|
| **Code** | 33177 |
| **Name** | Integrated internship methods |
| **Cycle** | Grade |
| **ECTS Credits** | 4.5 |
| **Academic year** | 2021 - 2022 |

**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 |
|---|---|
| PEÑARRUBIA BLASCO, DOLORES | 30 - Biochemistry and Molecular Biology |
| SENDRA PEREZ, RAMON | 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 intended for students to know, contact and become familiar with those experimental techniques of biochemistry and molecular biology more common today. It will also try the student to develop specific practical skills indispensable in empirical scientific discipline. The course introduces students instrumental in managing basic biochemistry laboratory and molecular biology, in obtaining physical and chemical parameters of biomolecules and the interpretation of those 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). This proposed a series of experiments related to the thematic order of most of the lessons of the theoretical program. In the Integrated Practice Methods course, purely practical type, 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 10 sessions with a total of 42 hours to be performed in two blocks (five sessions each). The first of these, consisting of 5 sessions to perform in different weeks (non-intensive) includes experiments spectrophotometry, spectrofluorimetry and diverse applications. The experiences made in this first block correspond to the theoretical contents explained in the first term in

the subject of theory, Methods in Biochemistry and Molecular Biology. The second block (5 sessions) to perform intensively in a week, including experiments and separation techniques and purification, whose theoretical basis is explained in the second term in the subject of theory, 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 teaching 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 five practices to be developed in five sessions (4 hours) in different weeks (not intensive)

Practice 1: 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.

Practice 2: Measurement of enzyme activities by spectrophotometry. Determination of specific activity and kinetic parameters of enzymes.

Practice 3: Analysis of metabolites in food enzyme using colorimetric methods. Enzymatic spectrophotometric determination of ethanol concentration.

Practice 4: protein-ligand interaction followed by fluorimetry. Management spectrofluorimeter. Excitation and emission spectra. Using ANS as fluorophore sensor polarity. Analysis of protein-ligand interaction.

Practice 5: Determination espectrofluorimétrica of Ca2+ and pH in solutions. Analysis of the variation of the excitation spectra of fluorophores QUIN2 and 5'-carboxy-4',5'-dimetilfluoresceína with Ca2+ concentration and pH, respectively. 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 4 hours, intensive 1 week), using chromatographic methods, electrophoretic and basic centrifuge. Application to the purification, characterization and analysis of Rubisco protein.

Practice 6: 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. The teacher will provide the student with advance a booklet / guide that will contain not only the protocols to follow but also references and a few issues that the student must solve (using literature) before practice. This will ensure that students have basic knowledge for the development and use of practical tasks.

The practical sessions will be raised so that the students participate and take charge of, if not all, most of the work required to perform the experiments: from the design of the experience, practice development, obtaining data and the preparation and interpretation of the results to finally provide a conclusion of the experiment. All this in the teaching laboratory and under the supervision of the teacher and working together with peers. At the end of practice (after each part) with the results and conclusions drawn students prepare and submit a technical report also to demonstrate its ability to formalize and communicate scientific data.

## EVALUATION

Part I (Practice 1-5) and Part II (Practice 6): 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 parties and a 5 in total to pass the course. Compensable notes 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 the two sides.

## REFERENCES

## Basic

- Primera parte (prácticas 1-5)

-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
-Walker, J.R.L. (1992) "Spectrophotometric determination of enzyme activity: alcohol dehydrogenase (ADH)". Biochem. Educ., 20, 42-43.

- Segunda parte (práctica 6)

-Andrews, T.J. y Lorimer, G.H. en The Biochemistry of Plants Vol. 10, 132-211. (Hatch, M.D. y Boardman, N.K. eds.) Acad. Press, 1987.
-Bradford, M. (1976). Anal. Biochem. 72, 248-254.
-Ellis, R.J. (1979). Trends Biochem. Sci. 4, 241-244.
-García-Martínez, J.L. y Moreno, J. (1986). Physiol. Plant. 66, 377-383.
-Hames, B.D. y Rickwood, D. Gel electrophoresis of proteins. A practical approach. IRL Press, 1981
-Keys, A.J. y Parry, M.A. en Methods in Plant Biochemistry Vol. 3, 1-14. (Dey, P.M. y Harborne, J.B. eds.) Academic Press. 1990.
-Lilley, R.McC., Walker, D.A. (1974). BBA, 358, 226-229.
-Peñarrubia, L., Moreno, J., Carrasco, P. (1988). Biochem. Educ., 16, 234-236.
-Saleemuddin et al. (1980). Anal. Biochem. 105, 202-205
-Schneider, G., Lindqvist, Y. y Brändén, C.I. (1992). Rubisco: structure and mechanism. Annu. Rev. Biophys. Biomol. Struct. 21, 119-143.

## ADDENDUM COVID-19

**This addendum will only be activated if the health situation requires so and with the prior agreement of the Governing Council**

**1. Contents**.

The contents initially collected in the GD are maintained.

**2. Volume of work and methodology.**

The proposed original work volume is maintained, but 50% distributed between in-person and non-face activities.

Students come to the laboratory in subgroups of 8 students (and not 16) and only in the half of the sessions. Activities of "data processing and virtual experimental results" are proposed (graphic representations, resolution of problems and questions, interpretation of results and discussion of the activities) for the remaining 50% of the time spent by the students.

For the face-to-face sessions, the practical laboratory activities have been adapted, merging experiments and simplifying them, as well as with the help of the teaching staff to carry them out. For the distance activities, new materials have been developed with experimental results and questions to be solved, as well as spoken presentations providing the pertinent explanations. Audio-visual material has also been prepared for those experiments or sections that are not carried out in the laboratory. In this way, the teaching objectives set out in these laboratory practices are largely covered.

**3. Evaluation.** For the evaluation, the score obtained in the proposed questionnaires and participation in the development of the practices and the score obtained from a written exam, carried out in person, will be considered. The distribution of the scores will be the one that appears in the original GD: 20% of Questionnaires and participation and 80% of the written exam.

**4. Bibliography.** There are no changes.

**5. Observations.** The distribution of teaching and the relationship between face-to-face and non-face-to-face activities may be modified throughout the course if sanitary conditions so require. Likewise, the exam would be carried out remotely by telematic means through the Virtual Classroom, if necessary due to the socio-sanitary situation of the moment.