VNIVERSITAT DE VALÈNCIA

Course Guide
33176 Methods in biochemistry and molecular biology

COURSE DATA

Data Subject	
Code	33176
Name	Methods in biochemistry and molecular biology
Cycle	Grade
ECTS Credits	12.0
Academic year	2021 - 2022

Study (s)

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

Subject-matter

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

Coordination

Name	Department
CARRASCO SORLI, PEDRO MIGUEL	30 - Biochemistry and Molecular Biology
MARTINEZ PASTOR, M TERESA	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 currently has, a large impact on the advance of biotechnology. This course addresses the specific tools and skills needed for experimentation in Biotechnology. The course introduces students to the fundamental basic methodologies and their applications in the field. The program for Methods in Biochemistry and Molecular Biology has been prepared for teaching second-year students in Biotechnology, and is not intended as a definitive proposal since the development of new techniques or the modification of the existing ones may advise their incorporation into the agenda. Each topic covers a tecnique or a set of related techniques. The topics are exposed beginning with a brief introduction to the physical foundation of the method (or group of methods), followed by a discussion of the experimental uses and applications. A significant number of application examples from different research fields have been selected based on their practical importance and their pedagogical value.

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 (RD 1393/2007) // LEARNING OUTCOMES (RD 822/2021)

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.
- Be able to perform an integrated analysis of gene expression at the level of transcriptome, proteome and metabolome.

LEARNING OUTCOMES (RD 1393/2007) // NO CONTENT (RD 822/2021)

· To design protocols for separation, purification and characterization of biological molecules

· To learn to use the basic equipment of a biochemistry and molecular biology laboratory

· To know the methodological bases of the techniques employed in molecular studies

· To acquire a basic knowledge of the main research techniques and methods

· To become familiar with the bibliographical sources which allow to find, select, understand and analyze the scientific information

· To understand and evaluate the methodology used by other scientist as published in research papers

· To discuss scientific papers in public

· To acquire the capacity to obtain methodological information from the bibliography, and to comment it with other students participating in the classroom discussion.

DESCRIPTION OF CONTENTS

1. Item 1. Characterization of Molecules of Biological Interest

1. The study of vital phenomena. Experiences in vivo and in vitro
2. Extraction and purification of biological molecules
2.1. Disorganization of living matter
2.1.1. Total and limited homogenization
2.1.2. Characteristics of the extraction medium. Protective agents

2.2. Purification of macromolecules
2.2.1. Preliminary separations. Precipitation and dialysis.
2.2.2. Preparative and analytical separation methods. Resolution
2.2.3. Yield and purification factor.
3. Levels of characterization of biomacromolecules
3.1. Characterization of proteins
3.2. Characterization of nucleic acids
3.3. Methods of identification and information. Specificity, accuracy, precision and sensitivity

2. Item 2. Absorption Spectroscopy

1. Radiation-matter interaction
1.1. Nature of the electromagnetic radiation
1.2. Molecular forms of energy. Quantum restrictions and Boltzmann distribution
1.3. Absorption of radiation. Chromophoric groups
2. Absorption spectroscopy. Overview
2.1. Measurement of absorption. Key components of a spectrophotometer
2.2. Lambert-Beer Law
2.2.1. Absorbance and extinction coefficient
2.2.2. Deviations. Isosbestic point
2.2.3. Study of mixtures of chromophores.
3. Spectroscopy in the infrared region (IR)
3.1. Bond vibration spectra
3.1.1. Bonds as harmonic oscillators
3.1.2. IR spectrophotometry. Sample Preparation
3.2. Biochemical applications
3.2.1. Typical absorption bands of nucleic acids and proteins
3.2.2. Deuterium-protium exchange
3.3.3. IR dichroism
3.3. Other forms of vibrational spectroscopy (FTIR and Raman)
4. Spectroscopy in the ultraviolet-visible región (UV-V)
4.1. Electronic spectra
4.1.1. Electronic jumps. Chromophore groups in the UV-V
4.1.2. UV-V spectrophotometry. Features
4.2. Biochemical applications
4.2.1. Protein absorption in the UV-V. Perturbation difference spectroscopy 4.2.2. Nucleic acids absorption in the UV-V. Hyperchromic effect
4.2.3. Determination of enzyme activities. Coupled reactions. artificial substrates
4.2.4. Colorimetry. Quantification of proteins

3. Item 3. Fluorescence Spectroscopy

1. Dissipation of energy by excited molecules
1.1. Radiant and non-radiative processes. Fluorescence and phosphorescence 1.2. Structural characteristics of the fluorescent compounds
2. Fluorescence spectroscopy. Overview
2.1. Parameters characterizing the fluorescent emission
2.1.1. Average life in the excited state and quantum yield
2.1.2. Stokes shift. Effect of the environment
2.2. Measuring fluorescence
2.2.1. Apparatus. Excitation and emission spectra. Corrected spectra
2.2.2. Relationship between fluorescence intensity and concentration
2.2.3. Quenching. Types of quenchers. Stern-Volmer equation
2.2.4. Temporal resolution of the fluorescence
2.3. Utility compared to absorption spectroscopy
3. Biochemical applications
3.1. Intrinsic and extrinsic fluorescence of proteins, nucleic acids and membranes
3.2. Determination of enzyme activities. Luminescence
3.3. Fluorescence polarization. Application to the study of membranes
3.4. Resonance induced energy transfer (FRET). Measuring molecular distances
3.5. Cellular studies
3.5.1. Fluorescence microscopy. Immunofluorescence
3.5.2. Cytometers and fluorescence-activated cell sorters
3.5.3. Measurement of intracellular Ca2 + and pH

4. Item 4. Nuclear Magnetic Resonance Spectroscopy

1. Nuclear magnetic resonance (NMR). Overview
1.1. Nuclear magnetic moment. Quantization under an external field
1.2. The NMR experiment. Apparatus
1.3. NMR spectra. Features
1.3.1. Chemical shift
1.3.2. Spin-spin coupling.
1.3.3. Longitudinal and transverse relaxation. Width and intensity of bands
1.3.4. Pulse sequences. Multidimensional NMR.
2. Applications
2.1. In vitro studies
2.1.1. Structure and dynamics of macromolecules and membranes
2.1.2. Determination of enzyme activities and binding of ligands
2.2. In vivo studies
2.2.1. Determination of intracellular pH and metabolites
2.2.2. Study of metabolic pathways
2.2.3. Imaging

5. Item 5. Mass spectrometry

1. Introduction. Physical foundation.
2. Mass spectrometer
2.1. Volatilization and ionization of samples.
2.1.1. Direct volatilization
2.1.1.1. Electron impact ionization (EI)
2.1.1.2. Chemical ionization (CI)
2.1.2. Matrix-assisted volatilization and ionization
2.1.2.1. Atom bombardment (FAB)
2.1.2.2. Plasma(PDI) and laser (MALDI)desorption
2.1.2.3. Electrospray (ESI) and ion spray (ISI)
2.2. Acceleration, focusing and detection of ions
2.2.1. Guided trajectory spectrometers
2.2.2. Cyclotron resonance devices (ICRMS)
2.2.3. Time of flight (TOF) spectrometers
2.3. Mass spectra
2.3.1. Intensity, accuracy and resolution.
2.3.2. Isotopic variability. Monoisotopic and average mass.
3. Biochemical applications:
3.1. Identification of organic compounds of small molecular mass
3.2. Study of proteins
3.2.1. Protein identification
3.2.2. Sequencing of Proteins and Peptides
3.2.3. Detection of posttranslational modifications
3.2.4. Isotopic labeling experiments in proteomics.

6. Item 6. Isotope methods

1. Fundamental principles of the use of isotopes in biochemistry
2. Radioactive decay
2.1. Types of emission. ß spectrum
2.2. Decay kinetics
2.3. Radioactivity units. Specific radioactivity
3. Detection and quantification of radioactivity
3.1. Gas ionization
3.1.1. Ionization chambers
3.1.2. Proportional counters
3.1.3. Geiger-Muller
3.2. Scintillation counters
3.2.1 Liquid Scintillation
3.2.1.1. Pulse height analyzers
3.2.1.2. Quenching
3.3.1.3. Cerenkov counting
3.2.2. Solid scintillation counter

3.2.3. Statistics of radioactive counting
3.3. Secondary induction of chemical reactions: Photographic detection.
4. Autoradiography
4.1. Overview
4.1.1. Autoradiographic emulsion. Characteristics of autoradiographic traces.
4.1.2. Resolution, efficiency, background
4.2. Autoradiographic methods
4.2.1. Temporary contact methods. Amplifying screens. Fluorography
4.2.2. Permanent contact methods.
4.3. Molecular autoradiography
4.4. Autoradiography for electron microscopy
4.5. Alternatives to autoradiograph: image surface detectors
4.5.1. Surface electronic detectors ("InstantImager")
4.5.2. Photostimulable image detectors ("FosforoImager")
5. Use of radioisotopes in biochemical research
5.1. In vivo studies
5.1.1. Rate of conversión and turnover time of a metabolite. Isotope dilution
5.1.2. Study of metabolic sequences. Pulses of radioactivity. Precursor-product relationships
5.1.3. Study of transport through membranes
5.1.4. Methods of double-labeling
5.2. In vitro studies
5.2.1. Enzymatic determinatios and study of reaction mechanisms
5.2.2. Isotopic exchange
6. Non-radioactive heavy isotopes
6.1. Percentage in excess of a heavy isotope. Detection
6.2. Heavy isotopes in quantitative proteomics

7. Item 7. Electrophoresis

1. Introduction: Background and definitions
1.1. Electrophoretic parameters: mobility
1.2. Free and zone electrophoresis
2. Non-limiting media electrophoresis
3. Protein electrophoresis in restrictive media
3.1 Polyacrylamide gel electrophoresis (PAGE)
3.1.1. Staining of polyacrylamide gels
3.1.2. Estimation of molecular masses: representation of Ferguson
3.1.3. PAGE discontinuous system
3.2 PAGE under denaturing conditions
3.2.1. SDS-PAGE
3.2.2. Estimation of molecular weights of proteins by SDS-PAGE
4. Isoelectric focusing
5. Two-dimensional electrophoresis
6. Electrophoresis of Nucleic Acids
6.1. Agarose gels

6.2. Methods of nucleic acid staining. Transfer to Membranes
6.3. Pulsed field electrophoresis
7. Capillary electrophoresis

8. Item 8. Chromatography

1. Introduction. Definitions, general and nomenclature
2. Background and classification of chromatographic methods
3. Paper chromatography
4. Thin layer chromatography (TLC)
5. Column chromatography: principles and basic operations
5.1. Chromatographic parameters
5.2. Capacity and resolution
5.2.1. Selectivity
5.2.2. Efficiency, the concept of theoretical plate
6. Adsorption chromatography
6.1. Ion exchange chromatography
6.1.1. Types of ion exchangers
6.1.2. Elution forms
6.1.3. Applications of ion exchange chromatography
6.1.4. Chromatofocusing
6.2. Hydrophobicity chromatography
7. Affinity Chromatography
7.1. Preparation of the stationary phase
7.2. Elution mechanisms
7.3. "Artificial" affinity chromatography
7.3.1. Chromatography on immobilized metals-IMAC
7.3.2. Chromatography on immobilized dyes
8. Other types of affinity chromatography. Chromatography on hydroxyapatite
9. Size exclusion chromatography
9.1. Background: the principle of separation
9.2. Applications
10. High Resolution Liquid Chromatography-HPLC
10.1. Instrumentation
10.2. Stationary phase: column types
10.3. Reverse phase HPLC RP-HPLC
10.4. Other types of HPLC.
10.4.1. UPLC.
10.4.2. Monolithic HPLC
11. Magnetic chromatography matrices

9. Item 9. Centrifugation

1. Introduction:
1.1. Background and definitions
1.2. Centrifugation theory
2. instrumentation
2.1. Types of Centrifuges
2.2. Types of Rotors
2.3. Preparative and analytical ultracentrifuge
3. Centrifugation methods
3.1. Differential centrifugation: Applications
3.2. Zonal centrifugation
3.2.1. Density gradient centrifugation
3.2.2. Isopycnic centrifugation
3.2.3. Gradient fractionation
3.2.4. Applications
3.3. Special preparative rotors
4. Analytical ultracentrifugation
4.1. Centrifuges, rotors and analytical centrifugation cells
4.2. Applications
4.2.1. Determination of sedimentation coefficients
4.2.2. Determination of molecular mass

WORKLOAD

ACTIVITY	Hours	% To be attended
Theory classes	86,00	100
Classroom practices	30,00	100
Tutorials	4,00	100
Preparation of evaluation activities	34,00	0
Preparing lectures	86,00	0
Preparation of practical classes and problem	60,00	0
TOTAL	**300,00**	

TEACHING METHODOLOGY

The course is devised to promote active learning of students. For this reason the lectures are intended as general introductions to each topic which will explain the research techniques and try to give a comprehensive and interrelated view of them. Prior to the lectures, the students will have bibliographic information and material provided by the teacher. It is intended that these classes have a very active participation of the students. Problems will be proposed to the students. Some problems will be given to them to solve in the classroom under the supervision of the teacher and working together with other students. During the whole-class comment of articles, students will participate in a discussion led by the

teacher, who will provide a research paper and a series of questions about the objectives, methodology, results and conclusions of the article.

EVALUACIÓN

There will be two written tests (partial) on the contents of the teaching activities. Each partial exam will consist of two parts: theory (5,5 points) and problems (4,5 points). The two parts must be surpassed independently to succeed the matter although the qualification of the parts will be compensable if it is higher than 4.5.

REFERÈNCIES

Bàsiques

- A. Textos que cubren la totalidad o la mayor parte de la asignatura

-Barceló, F. Técnicas Instrumentales en Bioquímica y Biología. Collecció materials didactics. Ed. Universitat de Les Illes Balears, 2003
-Creighton, T.E. The Physical and Chemical basis of Molecular Biology, Helvetian Press, 2010
-Cooper, T.G. "Instrumentos y técnicas de bioquímica" Ed. Reverté, 1984
-Freifelder, D. "Técnicas de bioquímica y biología molecular" Ed. Reverté, 1979
-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
-García Segura, J.M. Espectroscopía in vivo por resonancia magnética Ed., 1991
-Holme, D.J. y Peck, H. "Analytical Biochemistry" 3th edition. Ed. Prentice Hall, 1998
-Roca, P., Oliver, J. y Rodriguez, A.M. Bioquímica: técnicas y métodos Ed Hélice. 2004.
-Scopes, R.K. "Protein purification" 2a ed. Springer Verlag, 1987
-Serdyuk, I.N., Zaccai, N. Zaccai, J. Methods in molecular biophysics Ed. Cambridge University Press, 2007.
-Sheeham, D. Physical biochemistry: Principles and applications 2nd edition. Ed. Wiley Blackwell, 2009.
-Wilson, K y John Walker, J. (Eds) Principles and Techniques of Biochemistry and Molecular Biology. Ed. Cambridge University Press. 2006