

In situ molecular techniques (MInvB) Guía docente 2024-25

INTRODUCTION

Subject description: The aim is to familiarize students with the theoretical basis and practical application of "in situ" molecular techniques. For this purpose, the students will perform immunocytochemistry, "in situ" hybridization and "in situ" detection of apoptosis. Students are expected to learn how to interpret the results of these techniques in order to apply them in their research

Degree: Master's Degree in Biomedical Research

Module: Module III- Supplementary training

Number of credits (ECTS): 2

Calendar description: First semester

Subject type: Optional

Professors:

Dr. M. Ángela Burrell (Course Coordinator) mburrell@unav.es

Dr. Marina Martín Rodríguez (Practicals Coordinator) mmartinr.1@unav.es

Departament: Pathology, Anatomy and Physiology

School of Medicine and School of Sciences

Classroom:

Schedule:

Language: English

COMPETENCES

Basic competences

• BC6: Possess and understand knowledgeable facts that serve as a basis or opportunity for being original in the development and/or application of ideas, frequently within the context of research.

• BC7: The students will be able to apply acquired knowledge and problem solving abilities to fields outside this program, including that which is new and scarcely known, within a more ample or multidisciplinary context related to the research, development and innovation of drugs.



• BC8: The students will be able to integrate concepts and manage the complex task of drawing valid conclusions from information which, in spite of being incomplete or limited, includes reflections regarding social and ethical responsibilities linked to the application of general knowledge, specific concepts and common sense to the research, development and innovation of drugs.

• BC9: The students will learn to relay their conclusions -and the most recent facts and reasoning supporting said conclusions- to specialized personnel and to the general public in a clear and precise manner.

• BC10: The students will have acquired learning abilities that will permit them to continue studying in a self-directed and autonomous manner.

General competences

• CG1: Ability to deal with biomedical challenges in depth, from different viewpoints, identifying the state of present-day science.

• CG2: Identification of significant questions or hypotheses regarding biomedical issues or problems and definition of the steps necessary to resolve such questions.

• CG3: Possession of creative ability and originality in order to be able to respond to the questions raised in biomedical research.

• CG4: Ability to select and use appropriate techniques in order to efficiently and accurately carry out biomedical research work.

• CG5: Possession of technical ability to obtain precise and reproducible results which can be used to draw valid and objective conclusions in the field of biomedicine.

• CG6: Possession of critical ability, both when reading scientific biomedical literature and when interpreting the results of experiments.

• CG8: Ability to write correct, precise and well-structured texts about different types of biomedical research work.

• CG9: Ability to work in a team with allocated tasks and participate in work meetings, contributing to the solution of biomedical problems and achievement of working group objectives.

Competences of the speciality complement

• CEE1: Understand the connection that exists between different knowledge areas in order to open up horizons with a view to addressing a biomedical research project with creativity.

• CEE2: Look into specific aspects that permit increasing the knowledge acquired in the speciality.

• CEE3: Acquire specific technical bases to understand scientific literature and/or to execute the research project.

PROGRAM



Part I. Immunohistochemistry

Theoretical program

1. Introduction to immunohistochemistry. Definition. Requisites.

2. General aspects of the antibodies. Polyclonal and monoclonal antibodies: advantages and disadvantages of their use.

3. Tissue processing for immunohistochemical techniques: Fixation. Embedding. Cutting. Possible pretreatments.

4. Immunohistochemical detection methods: Direct and indirect methods. Immunofluorescence. Immunoenzymatic methods: with peroxidase or alkaline phosphatase. Methods with avidin-biotin. Methods with colloidal gold. Multiple staining methods.

5. Specificity of immunohistochemical techniques: Definition of specificity. Factors that can produce non-specific staining. Methods to verify the specificity: Positive and negative controls.

Practical program

- Double immunohistochemical method: Identification of two antigens on sections of paraffin-embedded material.

Part II. In situ hybridization and detection of apoptosis

Theoretical program

- 1. Introduction: guidelines for *in situ* hybridization (ISH).
- 2. General aspects of nucleic acid hybridization.
- 3. Clinical applications of ISH.
- 4. Details of the technique: probe synthesis and labeling.
- 5. Procedures of ISH to tissues.
- 6. Controls of the technique.
- 7. Apoptosis: General aspects and clinical implications.
- 8. Methods for detection of apoptotic cells: TUNEL assay.
- 9. Controls of the TUNEL assay.

Practical program

- Identification of EGFR gen amplification using fluorescence *in situ hibridization* technique (FISH).

- Detection of apoptotic cells by TUNEL assay.

EDUCATIONAL ACTIVITIES



This is a subject of 2 ECTS (50 h). The work is distributed as follows:

1. Classroom activities (0,84 ECTS, 21 h)

Part I: Immunohistochemistry: 0.32 ECTS (8 h)

- Lecture format 0.08 ECTS (2 h)

- Laboratory practice 0.24 ECTS (6 h)

Part II. In situ hybridization and detection of apoptosis: 0.48 ECTS (12 h)

- Lecture format 0.08 ECTS (2 h)

- Laboratory practice 0.40 ECTS (10 h)

Final exam: 0.04 ECTS (1 h)

2. Personal work (1,16 ECTS, 29 h)

- To make a laboratory notebook following the guidelines provided 0.52 ECTS (15 h)

- Personal study 0.48 ECTS (13 h)

ASSESSMENT

ORDINARY CALL

SPECIAL CALL

HORARIOS DE ATENCIÓN

Course director: Dr. Marián Burrell

Departament of Pathology, Anatomy and Physiology

Research Building (Edificio de Investigación), 3rd floor, Office 3370

Please make an appointment with Dr. Marián Burrell mburrell@unav.es

BIBLIOGRAPHY AND RESOURCES



Fundamental bibliography

Técnicas en Histología y Biología Celular. L. MONTUENGA, F.J. ESTEBAN, A. CALVO. Elsevier-Masson 2014; 2ª Edición. CIT.(5) 001.175 ; S.CIT. (5) 001.007 Find it in the Library (e-book)

Further reading for Part I: Immunohistochemistry

Nguyen, T. (Ed.), 2022, "Immunohistochemistry: A Technical Guide to Current Practices". Cambridge: Cambridge University Press. doi:10.1017/9781009106924 <u>Find it in the Library</u> (ebook)

Immunohistochemistry. Basics and Methods. BUCHWALOW IB y BÖCKER W. Springer Berlin Heidelberg, 2010 Find it in the Library (e-book)

Atlas de inmunohistoquímica. MARTÍN-LACAVE I y GARCÍA-CABALLERO T. Diaz de Santos, 2012 Find it in the Library (e-book)

Theory and practice of histological techniques. BANCROFT JD y GAMBLE M (eds). Churchill Livingstone, Edinburgh, 2008 (6^a ed.) Find it in the Library

Further reading for Part II: "In situ" hybridization and apoptosis

Immunocytochemistry and in situ hybridization in the biomedical sciences. JULIAN E. BEESLEY, editor. Boston [etc.] : Birkhäuser, cop. 2001. CIT.(4) 001.199 Find it in the Library

In situ hybridization: laboratory companion. Melody Clark (ed.) London [etc.] : CHAPMAN AND HALL, 1996 GEN.(2) 001.382 Find it in the Library

In situ hybridization: a practical approach. edited by D.G. WILKINSON Oxford [etc.] : IRL Press, 1998 Find it in the Library (e-book)

In situ hybridization: a practical guide. A.R. LEITCH. [et al.] Oxford : BIOS Scientific, 1994 CIT. (5) 001.108 Find it in the Library

Nucleic acid hybridization: essential techniques. Edited by J. ROSS New York [etc.] : John Wiley & Sons, 1997 Find it in the Library

A Simplified In Situ Hybridization Protocol Using Non-radioactively Labeled Probes to Detect Abundant and Rare mRNAs on Tissue Sections; <u>https://www.roche-applied-science.com</u> /PROD_INF/BIOCHEMI/no1_98/p10.pdf