[Medicinski fakultet u Rijeci]

Curriculum
2025/2026

[Za kolegij]

Methods of Protein Immunoanalysis

Study program:	**Medical Studies in English (R)** (elective) [Sveučilišni integrirani prijediplomski i diplomski studij]
Department:	**[Centar za proteomiku]**
Course coordinator:	**prof. dr. sc. Lenac Roviš Tihana**
Year of study:	**3**
ECTS:	**1.5**
Incentive ECTS:	**0 (0.00%)**
Foreign language:	**Possibility of teaching in a foreign language**

Course information:

overview:

L1 (2h) Lecture: Antibody Structure and Multiple Staining Experimental Design

In this introductory lecture, we will explore the basic characteristics of antibodies and delve into the design of experiments involving multiple staining. The structure of antibodies will be covered, along with techniques for labeling antibodies using fluorophores, biotin, and enzymes. Through several examples, we will highlight the crucial considerations when performing experiments that incorporate multiple staining.

L2 (1h) Lecture: Bioinformatics Tools for Protein and Antibody Structure Analysis

Discover the world of bioinformatics tools for analyzing protein and antibody structures. This lecture will focus on the three-dimensional representation of complexes, proteins, and antibodies, enabling students to gain insights into the advanced techniques used in structural analysis.

L3 (2h) Lecture: ELISA Kits for Protein Measurement in Research and Diagnostics

Explore the principles and applications of ELISA kits for protein measurement in research and diagnostic laboratories. This lecture will present an advanced laboratory experiment for determining protein analytes, such as soluble tumor markers or inflammatory marker proteins, using direct and indirect ELISA methods. We will also discuss more complex variations, including competitive ELISA. Furthermore, approved diagnostic kits, such as the immunoglobulin IgG test for determining SARS-CoV-2 antibody titers or food hypersensitivity, will be presented.

L4 (2h) Lecture: Western Blotting and Advanced Immunoprecipitation Methods in Research and Diagnostics

Discover the utility of Western blotting and advanced immunoprecipitation methods in medical research and diagnostics. We will explore how the high sensitivity and ability to detect proteins of different sizes make Western blotting a valuable tool in medical diagnostics. Examples will be provided, including its application in diagnosing borreliosis, Creutzfeldt-Jakob disease, and certain viral infections. Advanced materials and techniques, such as magnetic beads and immunoprecipitation from complex protein-rich samples like blood, will also be discussed.

L5 (1h) Lecture: Fundamentals of Flow Cytometry Analysis

Gain a solid understanding of the basics of flow cytometry analysis for protein immunoanalysis on cells, particularly surface protein analysis. This lecture will establish the foundation for interpreting flow cytometry results by covering histogram and dot blot presentations. Students will develop the necessary skills to comprehend literature in experimental medicine.

L6 (2h) Lecture: Immunofluorescence Microscopy with Confocal Analysis

Immerse yourself in the world of immunofluorescence microscopy, a critical method for analyzing proteins in cells and tissues. Building upon the principles of immunofluorescence and its application in confocal microscopy, this lecture will focus on designing advanced experiments and preparing for simple experiments involving multiple staining of cell culture preparations using fluorescently labeled antibodies. Additionally, advanced methods such as FRET (Fluorescence Resonance Energy Transfer) for protein interaction analysis and Two-photon excitation microscopy for thicker samples like organotypic models will be briefly explained.

E1 (1h) Experimental: Designing Experiments with Multiple Immunostaining

Engage in individual tasks aimed at finding suitable antibodies from online sources to immunofluorescently stain assigned proteins in a sample. This hands-on exercise will strengthen students' ability to design experiments involving multiple immunostaining.

E2 (5h) Experimental: Sample Preparation for Immunofluorescence Microscopy

Immerse yourself in the practical aspect of immunofluorescence microscopy by preparing samples (up to 4 colors) for staining on cell culture samples.

E3 (3h) Experimental: Analysis of Samples on Confocal Microscope

Experience the analysis of samples using a confocal microscope, a powerful tool in immunofluorescence microscopy. Students will gain practical skills in analyzing their prepared samples on the confocal microscope.

Seminars (6h): Engage in short student presentations with discussions, fostering an interactive learning environment where students can share their findings and engage in fruitful discussions.

The goal of the course is to help students deepen their newly acquired knowledge of protein immunoanalysis, that is, of protein analysis methods based on the use of antibodies. This type of analysis is the basis of many research and diagnostic procedures, and students will later be able to use their knowledge in, for example, clinical immunology, infectology, microbiology, oncology, pathology and molecular diagnostics. Basic knowledge in protein biology and immunological laboratory methods is expected, as well as a strong interest in knowledge in the field of molecular mechanisms in health and disease.

List of assigned reading:

Berg JM, Tymoczko JL, and Stryer L: Biochemistry Stryer – selected chapters

List of optional reading:

Selected scientific and review papers from the course topic

Curriculum:

[Predavanja] list (with titles and explanation):

L1 (2h) Basic characteristics of antibodies and design of experiments with multiple staining

The student will be able to:

Define the following terms related to protein immunoanalysis methods: primary and secondary antibodies, antibody cross-reactivity, antibody labeling, antibody origin (species), antibody isotype, diagnostic kit

L6 (2h). Immunofluorescence with confocal microscope analysis

The student will be able to:

1. Define the following terms related to immunofluorescence protein analysis methods: fluorophore, confocal, autofluorescence

L4 (2h) Western blotting and advanced immunoprecipitation methods in research and diagnostics

The student will be able to:

1. Discuss the advantages and limitations of the Western blot/immunoblot method in laboratory practice

L2. (1h) Bioinformatic tools for protein and antibody structure analysis

The student will be able to:

Describe the basic characteristics of protein-antibody complexes.

Locate tools that enable easy visualization of protein structures.

L5. (1h) Basics of analysis of cytometry results

The student will be able to:

1. Describes the basic results of flow cytometry and its advantage in the analysis of cell surface proteins

L3 (2h). ELISA kits for measuring proteins in research and diagnostic laboratories

The student will be able to:

1. Discuss the advantages and limitations of ELISA methods in laboratory practice

2. Discuss the factors that complicate the analysis of proteins in the blood

[Seminari] list (with titles and explanation):

S1 (1h) flow cytometry discussion and examples

Seminars (6h): Short presentations by students are foreseen with a discussion on the content of the course topic and relevant scientific literature or literature related to diagnostic procedures. The possibility of teamwork and incorporating the results obtained during solving experimental tasks.

Student obligations:

regular attendance of classes (lectures, seminars, exercises), preparing a seminar presentation/passing the final exam

Exam (exam taking, description of the written/oral/practical part of the exam, point distribution, grading criteria):

Other notes (related to the course) important for students:

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COURSE HOURS 2025/2026

Methods of Protein Immunoanalysis

[Predavanja] (Place and time or group)	[Seminari] (Place and time or group)
12.03.2026	
L1 (2h) Basic characteristics of antibodies and design of experiments with multiple staining: • [Centar za proteomiku] (09:00 - 10:30) [180] ○ MOPI	
prof. dr. sc. Lenac Roviš Tihana [180]	
18.03.2026	
L6 (2h). Immunofluorescence with confocal microscope analysis: • [Centar za proteomiku] (10:00 - 11:30) [180] ○ MOPI	
prof. dr. sc. Lenac Roviš Tihana [180]	
27.03.2026	
L4 (2h) Western blotting and advanced immunoprecipitation methods in research and diagnostics: • [ONLINE] (09:30 - 11:00) [180] ○ MOPI	
prof. dr. sc. Lenac Roviš Tihana [180]	
16.04.2026	
L3 (2h). ELISA kits for measuring proteins in research and diagnostic laboratories: • [P03 - INFORMATIČKA UČIONICA] (10:00 - 11:30) [180] ○ MOPI	
prof. dr. sc. Lenac Roviš Tihana [180]	

List of lectures, seminars and practicals:

[PREDAVANJA] (TOPIC)	Number of hours	Location
L1 (2h) Basic characteristics of antibodies and design of experiments with multiple staining	2	[Centar za proteomiku]
L6 (2h). Immunofluorescence with confocal microscope analysis	2	[Centar za proteomiku]
L4 (2h) Western blotting and advanced immunoprecipitation methods in research and diagnostics	2	[ONLINE]
L2. (1h) Bioinformatic tools for protein and antibody structure analysis	1	
L5. (1h) Basics of analysis of cytometry results	1	
L3 (2h). ELISA kits for measuring proteins in research and diagnostic laboratories	2	[P03 - INFORMATIČKA UČIONICA]

[SEMINARI] (TOPIC)	Number of hours	Location
S1 (1h) flow cytometry discussion and examples	1	

EXAM DATES (final exam):
