

Faculty of Medicine in Rijeka

**Curriculum  
2025/2026**

For course

**Methods of Protein Immunoanalysis**

Study program: **Medical Studies in English (R)** (elective)  
University integrated undergraduate and graduate study  
Department: **Centre for Proteomics**  
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:**

### **Practicals list (with titles and explanation):**

#### **E2(3h) Staining of samples (infected cells) for IF and confocal analysis**

Learning Outcomes

After completing this practical session, students will be able to:

- explain the basic principle and applications of immunofluorescence microscopy
- perform the main steps of an immunofluorescence staining protocol on cultured cells
- describe fluorescently labeled samples analysed by a fluorescence/confocal microscope

#### **E1(1h) Preparing antibodies and cells for IF and confocal analysis**

Learning Outcomes

After completing this practical session, students will be able to:

- explain the basic principle and applications of immunofluorescence microscopy
- perform the main steps of an immunofluorescence staining protocol on cultured cells
- describe fluorescently labeled samples analysed by a fluorescence/confocal microscope

#### **E3a(1h) preparing (tumor) tissues for immunohistochemistry**

Learning Outcomes

After completing this practical session, students will be able to:

- explain the basic principles and applications of immunohistochemistry
- perform the main steps of an immunohistochemical staining protocol on tumor tissue samples
- analyze and describe immunohistochemically stained tumor tissue samples using microscopy

#### **E3b(2h) immunohistochemical staining protocol**

Learning Outcomes

After completing this practical session, students will be able to:

- explain the basic principles and applications of immunohistochemistry
- perform the main steps of an immunohistochemical staining protocol on tumor tissue samples
- analyze and describe immunohistochemically stained tumor tissue samples using microscopy

#### **E3c(1h) analyze tumor tissue sample using microscopy**

Learning Outcomes

After completing this practical session, students will be able to:

- explain the basic principles and applications of immunohistochemistry
- perform the main steps of an immunohistochemical staining protocol on tumor tissue samples
- analyze and describe immunohistochemically stained tumor tissue samples using microscopy

### **Lectures 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

### **Seminars 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.

#### **S2 (1h) protein immunoanalysis, a structural perspective**

Ishodi učenja

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

<b>Lectures</b> (Place and time or group)	<b>Practicals</b> (Place and time or group)	<b>Seminars</b> (Place and time or group)
<b>12.03.2026</b>		
L1 (2h) Basic characteristics of antibodies and design of experiments with multiple staining: <ul style="list-style-type: none"><li>• Center for Proteomics (09:00 - 10:30) <sup>[180]</sup><ul style="list-style-type: none"><li>◦ MOPI</li></ul></li></ul>		
prof. dr. sc. Lenac Roviš Tihana <sup>[180]</sup>		
<b>18.03.2026</b>		
L6 (2h). Immunofluorescence with confocal microscope analysis: <ul style="list-style-type: none"><li>• Center for Proteomics (10:00 - 11:30) <sup>[180]</sup><ul style="list-style-type: none"><li>◦ MOPI</li></ul></li></ul>		
prof. dr. sc. Lenac Roviš Tihana <sup>[180]</sup>		
<b>27.03.2026</b>		
L4 (2h) Western blotting and advanced immunoprecipitation methods in research and diagnostics: <ul style="list-style-type: none"><li>• ONLINE (09:30 - 11:00) <sup>[180]</sup><ul style="list-style-type: none"><li>◦ MOPI</li></ul></li></ul>		
prof. dr. sc. Lenac Roviš Tihana <sup>[180]</sup>		
<b>16.04.2026</b>		
L3 (2h). ELISA kits for measuring proteins in research and diagnostic laboratories: <ul style="list-style-type: none"><li>• P03 - IT CLASSROOM (10:00 - 11:30) <sup>[180]</sup><ul style="list-style-type: none"><li>◦ MOPI</li></ul></li></ul>		
prof. dr. sc. Lenac Roviš Tihana <sup>[180]</sup>		
<b>24.04.2026</b>		
L5. (1h) Basics of analysis of cytometry results: <ul style="list-style-type: none"><li>• ONLINE (09:30 - 10:15) <sup>[198]</sup><ul style="list-style-type: none"><li>◦ MOPI</li></ul></li></ul>		S1 (1h) flow cytometry discussion and examples: <ul style="list-style-type: none"><li>• ONLINE (10:15 - 11:00) <sup>[198]</sup><ul style="list-style-type: none"><li>◦ MOPI</li></ul></li></ul>
izv. prof. dr. sc. Juranić Lisnić Vanda, dipl. ing. bioteh. <sup>[198]</sup>		
<b>30.04.2026</b>		
L2. (1h) Bioinformatic tools for protein and antibody structure analysis: <ul style="list-style-type: none"><li>• P08 (10:00 - 10:45) <sup>[192]</sup><ul style="list-style-type: none"><li>◦ MOPI</li></ul></li></ul>		S2 (1h) protein immunoanalysis, a structural perspective: <ul style="list-style-type: none"><li>• P08 (10:45 - 11:30) <sup>[180]</sup><ul style="list-style-type: none"><li>◦ MOPI</li></ul></li></ul>
prof. dr. sc. Lenac Roviš Tihana <sup>[180]</sup> . dr. sc. Lisnić Berislav, dipl. ing. <sup>[192]</sup>		
<b>08.05.2026</b>		

	<p>E1(1h) Preparing antibodies and cells for IF and confocal analysis:</p> <ul style="list-style-type: none"> <li>Center for Proteomics (08:00 - 08:45) [1625] [180] <ul style="list-style-type: none"> <li>MOPI</li> </ul> </li> </ul> <p>E2(3h) Staining of samples (infected cells) for IF and confocal analysis:</p> <ul style="list-style-type: none"> <li>Center for Proteomics (08:45 - 11:00) [2827] <ul style="list-style-type: none"> <li>MOPI</li> </ul> </li> </ul>	
dr.sc. Cokarić Brdovčak Maja [2827] · prof. dr. sc. Lenac Roviš Tihana [180] · Malić Suzana [1625]		
<b>14.05.2026</b>		
	<p>E3a(1h) preparing (tumor) tissues for immunohistochemistry:</p> <ul style="list-style-type: none"> <li>Center for Proteomics (08:00 - 08:45) [180] [1431] <ul style="list-style-type: none"> <li>MOPI</li> </ul> </li> </ul> <p>E3b(2h) immunohistochemical staining protocol:</p> <ul style="list-style-type: none"> <li>Center for Proteomics (08:45 - 10:15) [180] <ul style="list-style-type: none"> <li>MOPI</li> </ul> </li> </ul> <p>E3c(1h) analyze tumor tissue sample using microscopy:</p> <ul style="list-style-type: none"> <li>Center for Proteomics (10:15 - 11:00) [180] [1412] <ul style="list-style-type: none"> <li>MOPI</li> </ul> </li> </ul>	
prof. dr. sc. Lenac Roviš Tihana [180] · Miklić Karmela [1412] · Mikša Leonarda [1431]		

### List of lectures, seminars and practicals:

LECTURES (TOPIC)	Number of hours	Location
L1 (2h) Basic characteristics of antibodies and design of experiments with multiple staining	2	Center for Proteomics
L6 (2h). Immunofluorescence with confocal microscope analysis	2	Center for Proteomics
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	P08
L5. (1h) Basics of analysis of cytometry results	1	ONLINE
L3 (2h). ELISA kits for measuring proteins in research and diagnostic laboratories	2	P03 - IT CLASSROOM

PRACTICALS (TOPIC)	Number of hours	Location
E2(3h) Staining of samples (infected cells) for IF and confocal analysis	3	Center for Proteomics
E1(1h) Preparing antibodies and cells for IF and confocal analysis	1	Center for Proteomics
E3a(1h) preparing (tumor) tissues for immunohistochemistry	1	Center for Proteomics
E3b(2h) immunohistochemical staining protocol	2	Center for Proteomics
E3c(1h) analyze tumor tissue sample using microscopy	1	Center for Proteomics

<b>SEMINARS (TOPIC)</b>	<b>Number of hours</b>	<b>Location</b>
S1 (1h) flow cytometry discussion and examples	1	ONLINE
S2 (1h) protein immunoanalysis, a structural perspective	1	P08

**EXAM DATES (final exam):**

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