



UNIVERSITÀ
DEGLI STUDI DI MILANO-BICOCCA

SYLLABUS DEL CORSO

Introduzione alle Tecniche di Laboratorio

1920-2-E1301Q079

Aims

The aim of the course is to provide students with a basic knowledge of the most important techniques in use in the biochemical laboratory. Students are encouraged to develop a critical approach in the choice of different strategies in protein purification and enzyme activity assays.

1. Knowledge and understanding - students will learn the theory underlying the most common biochemical techniques, as well as the basis of the most important molecular and cell biology techniques. A particular emphasis will be set on the general principles underlying recombinant protein production, purification and characterization
2. Applying knowledge and understanding - at the end of the course students will be able to apply the knowledge acquired to the choice of the different experimental approaches in protein purification and characterization; this knowledge will also be essential in other courses, particularly in the "Integrated biological laboratory" course
3. Making judgements – at the end of the course students will be able to devise a protein purification protocol and to choose among the different characterization methods
4. Communication skills - students will acquire a specific scientific language and the ability to orally describe the topics discussed in the course
5. Learning skills - this course will provide students with the ability to understand and critically evaluate the experimental methods described in the scientific literature.

Contents

Cell disruption methods; centrifugation; UV-VIS spectroscopy and spectrofluorimetry; protein concentration and enzyme activity assays; chromatographies; strategies in protein purification; electrophoresis; production of recombinant proteins; immunochemical techniques; radioisotopic techniques; manometry; an overview of methods for primary structure analysis.

Detailed program

Cell disruption methods – Buffers and proteins stability; soft, medium and hard methods for cells disruption; salting out; dialysis

Centrifugation – general principles; differential centrifugation; density gradient centrifugation; elutriation

UV-VIS spectroscopy and spectrofluorimetry

Protein concentration and enzyme activity assay – Bradford and BCA assays; experimental setting for enzyme activity assays

Chromatography – general principles; gel filtration; ion-exchange chromatography; hydrophobic chromatography; chromatofocusing; reverse phase chromatography; affinity chromatography

Strategies in protein purification

Electrophoresis – general principles; SDS-PAGE; native electrophoresis; isoelectrofocusing; 2D electrophoresis

Production of recombinant proteins – vectors for the expression of tagged recombinant proteins; purification of tagged proteins and tag removal

Immunochemical techniques – immunoprecipitation; western blotting; ELISA; RIA; immunocytochemistry

Radioisotopic techniques – radioactivity; radioisotopes in use in the biochemical laboratory; Geiger-Muller counter; scintillation counter; autoradiography

Manometry – Clark and Rank electrodes

Methods for proteins primary structure analysis – aminoacid analysis; Edman degradation; mass spectrometry

Prerequisites

A basic knowledge of general and organic chemistry, physics and biochemistry is essential. The essential physico-chemical principles and biochemistry topics will be reviewed before addressing each method.

Teaching form

Front lectures based on powerpoint presentations; videos; active learning (discussion of experimental data)

Textbook and teaching resource

Suggested textbook:

Wilson&Walker "Biochimica e Biologia molecolare" Ed. Cortina (2019);Bonaccorsi, Contestabile, Di Salvo "Metodologie biochimiche" CEA

slides are available at Moodle site

Semester

Second semester

Assessment method

Oral examination: The oral examination is aimed at assessing the student's ability to illustrate coherently the course main topics and to discuss experimental data. It consists of 3-4 general questions.
No in itinere evaluations are scheduled.

Office hours

upon request (paola.fusi@unimib.it)
