

66026 - Cellular separation. Cellular viability study

Syllabus Information

Academic Year: 2019/20

Subject: 66026 - Cellular separation. Cellular viability study

Faculty / School: 100 - Facultad de Ciencias

Degree: 537 - Master's in Molecular and Cellular Biology

ECTS: 6.0

Year: 1

Semester: Second semester

Subject Type: Optional

Module: ---

1.General information

1.1.Aims of the course

1.2.Context and importance of this course in the degree

1.3.Recommendations to take this course

2.Learning goals

2.1.Competences

2.2.Learning goals

2.3.Importance of learning goals

3.Assessment (1st and 2nd call)

3.1.Assessment tasks (description of tasks, marking system and assessment criteria)

4.Methodology, learning tasks, syllabus and resources

4.1.Methodological overview

The learning process that has been designed for this course is based on the following:

- Lectures: 10 h
- Laboratory sessions 40 h
- Seminars and oral presentations: 10 h

Classroom materials will be available via Moodle. These include a repository of the lecture notes used in class, the course syllabus, as well as other course-specific learning materials, including the protocols for the laboratory sessions.

4.2.Learning tasks

The program offered to the student to help him achieve the expected results includes the following activities:

Lectures (10 h): In-class activity. They will take place in the morning. In them, the basic theoretical knowledge of the subject is presented.

Laboratory sessions (40 hours): In-class activity. They will take place in the Biology and Biochemistry lab, in groups of 8 people maximum. Students will work individually or in pairs. They will be interspersed with the theoretical classes, the students will put into practice what they learned from them.

Seminars (10 h): In-class activity. The students will perform an oral presentation based on the critical analysis of scientific literature, related to the methodology learned in the lectures and practical classes. The student will analyse of the methodology used and the results obtained, which will be presented in front of the rest of the students and professors. The

seminars will take place during two sessions on two different days (depending on the number of students).

4.3.Syllabus

The course will address the following topics:

1. Lectures

1. Introduction and general comments (the bibliography needed for preparing the seminars has already been assigned to each student previously)
2. Assessment of cell functionality
3. Cell separation by chemotaxis, *swim-up* and filtration techniques
4. Cell separation by centrifugation methods
5. Flow cytometry
6. Electrophoresis of cells
7. Cell affinity separation
8. Cell partition in aqueous two-phase systems

2. Practice sessions

1. Separation of motile cells: Swim-up versus Sucrose washing.

Comparison of the efficiency of both methods following:

- Cell counting
- Motility evaluation by CASA
- Cell viability assessment by fluorescence microscopy
- Cell viability assessment by flow cytometry

1. Cold-shock effect on cell functionality

a) Analysis of the initial sample determining:

- Viability (CFDA/PI staining) by fluorescence microscopy and flow cytometry
- Reactive oxygen species (ROS) by flow cytometry
- Capacitation state (spermatozoa) by CTC staining and fluorescence microscopy

b) Analysis of the cold-shocked sample determining:

- Viability post-*cold-shock* (CFDA/PI staining) by fluorescence microscopy and flow cytometry
- Reactive oxygen species (ROS) post-*cold-shock* by flow cytometry
- Capacitation state post-*cold-shock* (spermatozoa) by CTC staining and fluorescence microscopy

1. Identification of cell subpopulations by detection of specific proteins through indirect immunofluorescence

a) Sample preparation

- Fixation and drying
- Washes
- Blocking and incubation overnight with the primary antibody

b) Analysis

- Washes
- Incubation with the secondary antibody
- Mounting and observation

1. Cell separation by partition chromatography in aqueous two phase systems

- Determination of the partition ratio

- Assessment of total and viable cell recovering

3. Seminars

Each student must explain the background, objectives, main methods, results and discussion of the assigned article. A personal and critical explanation within the framework of the course is required. A written summary has also to be presented.

4.4.Course planning and calendar

Lectures and practice sessions will usually take place between 4th and 24th of March.

Further information concerning the timetable, classroom, assessment dates and other details regarding this course, will be provided on the first day of class or please refer to the virtual platform Moodle <https://moodle2.unizar.es/add/>,

4.5.Bibliography and recommended resources