



DISCOVERING CELL BIOLOGY: A BASIC COURSE IN CELL CULTURE HANDLING AND CELL VIABILITY ASSAYS

MONDAY

10:00 welcome.

10:30 lesson: cell culture handling. *Content: primary cell cultures and cell lines; composition and types of growth medium; temperature and the role of CO₂; control of sterility and types of consumables; adherent vs suspension cells.*

11:00 practice: how to split and count adherent and suspension cells. *Activity: students will be shown how to detach and count cells by using the Burker chamber.*

12:00 lesson: cell viability assays. *Content: assays that can be used to analyze cell viability; proliferation assays; quantification of metabolic activity; techniques to detect death cells; planning of a viability experiment by using the crystal violet assay.*

15:30-17:30 practical activity in small groups: splitting of adherent and suspension cells, counting and preparation of plates for treatments. *Activity: students will detach the cells, count them and prepare the plate for crystal violet and PI assay.*

TUESDAY

10:00: lesson: basic principles in flow cytometry. *Content: the flow cytometer; forward scatter and side scatter light; fluorescent channels, adsorption and emission spectra; the fluorochromes. How to set up an experiment.*

11:00-12:30 practical activity in small groups: treatment of adherent and suspension cells. *Activity: a specific drug and an effective concentration range will be assigned to each group. Students will calculate the amount of stock solution to be used for a treatment curve and which will be the appropriate controls. The teacher will add the drug according to student indications.*

14:30-16:30 practical activity: fixing and staining cells for cell cycle analysis. *Activity: students will fix and stain cells for cell cycle analysis*

WEDNESDAY

9:00 practice: crystal violet assay on adherent cells part 1. *Activity: students will wash, fix, stain the plates with crystal violet.*

10:30 practice in small groups: become familiar with the flow cytometer. *Activity: students will be shown the flow cytometer and illustrated the analysis software, the different types of graphs, the fluorescence channels and the compensation concept. Finally, an example of analysis of a sample colored for multiple markers will be shown.*

14:00 practice: crystal violet assay on adherent cells part 2. *Activity: students will destain the plates and read absorbance.*

15:30-17:30 practical activity in small groups: PI incorporation assay on suspension cells. *Activity: students will stain cells with PI and analyze data at the flow cytometer.*

THURSDAY

10:00 practical activity: analysis of crystal violet PI data and calculation of IC50. *Activity: Students will reorganize the crystal violet and PI data in an excel spreadsheet and will calculate IC50 value.*

11:00 practical activity in small groups: analysis of cell cycle at the flow cytometer. *Activity: students will analyze their own samples at the flow cytometer.*

14:00 evaluation test.