

Graduate Program in Molecular Cell Biology and Oncology
MCBO Methods Courses

Special Lecture/Course in: Key techniques:

Cell culture, lysis, fractionation and detection of specific proteins and their activity.

Lecturer: Ilja Vietor and Mariana Araujo

Number:

Type: VU Semester hours: 1,5

Character: Block

Time/Date: 18th or 22nd calendar week 2019

Location: Div. Cell Biology, Biocenter, Innrain 80, 2nd floor.

Limited number of places YES, number of places 6, registration necessary YES

For registration or questions please contact: ilja.vietor@i-med.ac.at

Aim:

Theoretical introduction and practical training on basic methods of subcellular fractionation; SDS PAGE; Western Blotting, cell transfection and luciferase activity measurement.

Description / contents:

Subcellular fractionation, allowing the separation of organelles based on their physical properties consists of two steps:

(1) disruption of the cellular organization (homogenization)

and

(2) fractionation of the homogenate to separate the different populations of organelles.

Each population of organelles is characterized by size, density, charge and other properties and purified fractions will be characterized by biochemical means.

Transcription factors fulfill their biological function in the nucleus. Nuclear translocation of a specific protein will be forced by treatment of cells and its activity detected using a reporter construct transfected into the cells.

Suggested reading:

Pasquali C, Fialka I, Huber LA. Subcellular fractionation, electromigration analysis and mapping of organelles. *J Chromatogr B Biomed Sci Appl.* 1999 Feb 5;722(1-2):89-102. Review.

De Araujo ME, Huber LA, Stasyk T, Isolation of endocytic organelles by density gradient centrifugation, *Methods Mol Biol.*2008: 424:317-31. Review

Vietor I, Kurzbauer R., Brosch G., Huber L.A.: TIS7 regulation of the beta-catenin / TCF-4 target gene OPN is histone deacetylase dependent. *J Biol. Chem.* 280: 39795-39801, 2005

Specific literature and protocols will be distributed via e-mail shortly before the course.