Brief Description

A well-designed research-based experimental laboratory course will be critical for students interested in research in biotechnology and related disciplines, and this course will help immensely. Theoretical principles of experimental techniques, procedures, assignments, and data analysis are important to understand the concept behind the experimental technique. This course is intended to familiarize students with the basics of some of the well-known experimental biotechnology science for their use and application in research. Students will gain experience in using biochemical instruments and techniques.

By attending this course, students will find the content useful in strengthening the basic skills in their field of interest and apply their learning to real-world problems. In addition, the book contains information on data analysis, statistics, units, safety, and best practices. This book is not only useful for a theoretical course, it can also be used to design research-based and hands-on courses, which will engage students in the learning of science skills focused on the solution of real scientific problems.

This course will be useful for seniors (undergraduate), graduate and PhD students. Prerequisite for this course is Biochemistry-I and Biochemistry-II.

A General Course Outline

Week 1: Introduction to the laboratory and literature search (Excercise I). Buffer preparation and titration of Tyr residues using pH meter (Excercise II).

Objective (Excercise I): Library literature search to obtain biochemically relevant information on glutathione-S-transferases.

Examples:

Group 1-Metabolic substrates of glutathione-S-transferases; *Group 2*-Effect of environmental factors on glutathione-S-transferases; *Group 3*-Role of glutathione-S-transferases in human or animal aging; *Group 4*-Plant senescence and glutathione-S-transferases.

Objective (Excercise II): Estimate pK of free Tyr residues. The results will be compared with the titration curve of a protein, (glutathione-S-transferase) to be determined in Exercise III.

Week 2: Protein estimation (Excercise IV)

Objective (Excercise IV): Use three different common methods of protein assay (Bradford, Lowry and spectroscopic) to determine absorption extinction coefficient of a protein, glutathione-S-transferase.

Week 3: Spectroscopic determination of protein pKa (Excercise III)

Objective (Excercise III): Demonstrate the use of absorption spectroscopy for pKa determination. Observe variation in the pKa of Tyr side chains in a protein (glutathione-S-transferase) compared to those in free aqueous solution. Calculate the number of ionized Tyr residues in glutathione-S-transferase.

Week 4: Discussion and catch-up

Week 5: Polyacrylamide gel electrophoresis (Excercise V)

Objective (Excercise V): Use sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to estimate molecular weight of a protein, glutathione-S-transferase.

Week 6: Isoelectric focusing of protein(s) (Excercise VII)

Objective (Exercise VII): Use isoelectric focusing-polyacrylamide gel electrophoresis (IEF-PAGE) to estimate pI of a protein, and classify the given glutathione-S-transferase.

Week 7: Size exclusion chromatography (Exercise VI)

Objective (Exercise VI): Determine the native molecular weight of a protein, glutathione-S-transferase, using size exclusion column

chromatography, and compare your results with the molecular weight obtained from SDS-PAGE.

Week 8: Ion-exchange chromatography (Exercise VIII)

Objective (Exercise VIII): Purification of different isotypes of glutathione-S-transferase using ion-exchange column chromatography. Obtain pure homogenous glutathione-S-transferase from quahogs or *Tetrahymena* (examples).

Week 9: Affinity chromatography (Exercise XI)

Objective (Exercise XI): Purification of glutathione-S-transferase using glutathione agarose affinity column chromatography. Obtain pure homogenous glutathione-S-transferase from oat plants or *Tetrahymena* (examples).

Week 10: Immunochemical technique-ELISA (Exercise IX)

Objective (Exercise IX): Examine the relationship between glutathione-S-transferases from two source with a polyclonal antibody using enzyme-linked immunosorbent assay (ELISA).

Week 11: Enzyme kinetics (Exercise X)

Objective (Exercise X): Estimate Km and Vmax of glutathione-S-transferase using glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB) as substrates.

Weeks 12-15: Isolation and biochemical characterization of glutathione-S-transferase from scallops, oats, quahogs or cranberries (examples).