

BIOC23H: Practical Approaches to Biochemistry Winter 2014

Description: A lecture and laboratory course that introduces students to experimental approaches used in biochemical research. Topics include practical and theoretical aspects of: spectrophotometry; chromatography, electrophoresis; radioisotopes; enzyme assay and protein purification. Students will be expected to solve numerical problems involving these and related procedures.

Prerequisites: Prerequisite: BIOB12H & BIOC12H Corequisite: BIOC13H

Lecture session:

Monday, 12:00-14:00, SW143

Instructor:

Dr. Rongmin Zhao, Office: SY248, Email: rzhao@utsc.utoronto.ca Please use E-mail ONLY when it is critical for you to get in touch with me and use BIOC23-Biochemistry in the subject line. Also your student name and student number must be included in your email.

Instructor office hours

Friday 2:00pm-4:00pm

Laboratory teaching assistant and lab coordinator

There is a teaching assistant for each lab section. Different TA may run the lab slightly differently and have different requirement for a particular lab. Students are required to follow his/her own TA's requirement and contact his/her own TA in case of any questions.

Course content

Lectures --- BIOC23 is a laboratory course. The material covered in the lecture will relate to the laboratory techniques carried out throughout the course. In the lecture, we will discuss methodologies, theories behind the methodology and relevant applications for the particular techniques used. Specific aspects of the protocols and the results of the labs will also be discussed. Lecture also gives you a chance to ask questions regarding the theory behind the labs you will be conducting. Therefore come with questions. Lecture material will be posted at least one day before the lecture.

There is no Textbook for this course. The lectures come from a number of sources including primary methodologies. The basic principle for some lab components has been discussed in the C12 course.

Laboratories --- **Attendance to labs is mandatory.** If you miss a lab, you must provide a UTSC medical certificate or other acceptable reason cleared by Dr. Zhao to be excused from the lab and to be able to hand in any assignments related to that lab. There is no possibility to makeup labs. Each missed lab will result in a loss of 4% of your grade. If you miss more than 3 laboratories without proper documentation, you will not be given credit for the lab component of the course. The labs are full and therefore you will not be able to makeup labs.

!!! Before coming to the laboratory, students are responsible for preparing the following items:

1. Lab coat (no exception) and closed toe shoes

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2. Safety glasses for most labs
3. A permanent black marker (Sharpie fine point)
4. A book for recording your work (your lab log book). This book can be hard or soft cover, or a binder. This book has to be used only for the C23 lab and will be collected at the end of the course.
5. A pen to record your lab and it is prohibited to use pencil to record your lab.

Supporting platform

This course will use the UofT Blackboard system as the supporting platform. All course related materials, announcements will be posted in the Blackboard system. You need to log in with your UTORid at the website <https://portal.utoronto.ca/webapps/portal/frameset.jsp>. You are responsible for checking the course website regularly for important information.

Laboratories are posted on the blackboard a week before the lab is scheduled. The emphasis of the course is the laboratory. The lecture complements the laboratory. **It is your responsibility** to come prepared each week to carry out your laboratory. To this end, before coming to the lab, you need to complete **an introduction** to each lab (a paragraph) explaining the purpose and to have written out your own **flow chart** based on the posted labs (this is what you should refer to when doing the lab). When carrying out experiments, you must take complete notes.

Therefore, as you do the experiment, you will write down in pen exactly what you did, what you have observed, such as times of incubations and any specific volumes, **AND ANY MISTAKE** you have done by accident.

You will have **data analysis** (including standard curve construction) **summary / conclusion**, where you analyze the data, do any graphs etc, which will be the required components of your lab assignment/report. To ensure you are prepared for the lab, there will also be **quizzes** before each lab topic. The quiz will test whether you have adequately prepared for the lab and will be an essential component for the final grade.

Equipment in the lab is expensive. You are responsible for your equipment. Accidents can happen but most “accidents” are avoidable with proper preparation and attention to the task.

Course Evaluation

Midterm (15%)

It will test the lab and lecture materials. There is no makeup term test. If you miss the midterm because of illness and provide me with the UTSC medical certificate filled in by your doctor, you will be eligible to combine the term test mark to the final exam. You must contact me within 48 hours in case that you miss the term test. The format of the midterm will be multiple choice and short answer questions.

Final exam (35%)

It will test the lab and lecture materials. The format of the final exam will be multiple choice and short answer questions. The final exam is cumulative.

Quizzes 12% (6X 2%)

Date of quizzes: Weeks of Jan 13, Jan 20, Jan 27, Feb 3, Feb 10, March 10, 2014)

Lab performance (10%)

Preparation (introduction and flow chart) 4% - The TA is going to check the introduction and flow chart.

Laboratory log / summary/data analysis and technical performance 6% (4% will be particular on ion exchange and gel filtration chromatography sessions)

Lab assignments (28%)

- 1. Mitochondrion isolation: (5%)** (Maximum of 2 pages of text not including figures/figure legends, tables and references and cover page)
- 2. Alkaline phosphatase, (6%)** will not require materials and methods requires brief introduction, graphs/with appropriate legends and data analysis (maximum 2 pages double spaced of text not including graphs, legends or references and cover page)
- 3. Formal report 1: IgG purification (8%)** (maximum 7 double spaced pages of text not including figures/figure legends/ reference page and cover page)
- 4. Formal report 2: GST-fusion protein isolation and Western blotting (9%)** (maximum 7 pages of double spaced pages of text not including figures/figure legends/ reference page and cover page)

The content required for each assignment will be explained during the appropriate lecture and laboratory class. It is also addressed at the end of each laboratory exercise.

Due dates:

The formal lab reports are due at the beginning of the lab indicated in the following weeks:

1. Mitochondrial isolation/differential centrifugation: week of Jan 20st, 2014
2. Alkaline phosphatase: week of Feb 10th, 2014
3. IgG purification: week of March 17th, 2014
4. GST and Western blotting with lab logbook: week of March 31st, 2014

Lecture schedule

Lectures relevant to the particular lab will be given either the week before or the week of the laboratory. Some topics will last more than one week.

Topic 1: pH and buffers

Topic 2: Cellular fractionation/

Topic 3: Enzyme kinetics

Topic 4: Protein isolation/purification

Topic 5: Chromatography

Topic 6: Protein quantification and gel electrophoresis

Topic 7: Immunochemistry/Immunoblotting analysis

Topic 8: Mass spectrometry and bioinformatics

Topic 9: Use of radioisotopes in biochemical studies

Laboratory Safety

- 1. Eating or drinking is prohibited in the laboratory.** Breaking this rule will lead to expulsion from the laboratory and loss of marks associated with the laboratory for which you have been asked to leave
- 2. Please do not bring** coats, hats, etc. into the laboratory.
- 3. You must Always wear a lab coat** (done up) in the laboratory with the sleeves rolled down and **closed shoes**

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4. Wear gloves or safety glasses when instructed to do so.
5. Keep paper, pencils, fingers, etc. out of your mouth.
6. At the beginning and end of the laboratory, follow procedure 12 and 13
7. Discard chemical waste in appropriate containers
8. Discard bacterial culture material in to autoclave bags
9. Discard pipettes point-down, in the upright plastic pipet holders. Make sure the pipet tips are covered with disinfectant.
10. Place all *test tubes* containing living cells in the designated racks/pans;
11. Discard pipette tips in the appropriately marked container
12. **Wash hands thoroughly with soap and water** once or twice during the lab, at any time you come in contact with l chemicals/live cells and also just before leaving the laboratory.
13. When leaving the lab, REMOVE YOUR LAB COAT. However, DO NOT UNDER ANY CIRCUMSTANCES wear your lab coat in any public area (hall way and washroom etc.)

Laboratory schedule for 2014 (Monday of the week is indicated)

Jan 6, 2014: Week 1

Find groups and lab benches. Lab safety issues. NO real lab components.

Jan 13, 2014: Week 2:

Experiment 1: Differential centrifugation/mitochondrial isolation

Jan 20, 2014: Week 3

Differential centrifugation assignment due

Experiment 2: Enzyme kinetics: alkaline phosphatase

Jan 27, 2014: Week 4:

Experiment 2: Data analysis

Experiment 3: Ion exchange chromatography

Feb 3, 2014: Week 5

Finish experiment 3: Thin layer chromatography

Experiment 4: Gel filtration

Feb 10, 2014: Week 6:

Enzyme kinetics: alkaline phosphatase lab report due

Experiment 5 part 1: IgG: salting out *via* ammonium sulfate/dialysis

Feb 17, 2014 reading week - no lab

Feb 24, 2014: Week 7:

Experiment 5 part 2: IgG: DEAE Sephadex and protein quantification

Mar 3, 2014: Week 8

Experiment 5 part 3: IgG: SDS gel electrophoresis and staining (**picture posted by the following day**).

Mar 10, 2014: Week 9

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Experiment 6 part 1: Purification of His₆-GST fusion proteins from *E. coli* (isolation and affinity chromatography)

Experiment 5 discussion of IgG data

Mar 17, 2014: Week 10

IgG report due

Experiment 6 part 2: His₆-GST fusion protein: gel electrophoresis and Western transfer

Mar 24, 2014: week 11

Experiment 6 part 3: His₆-GST fusion protein immunoblotting with anti-GST antibody/dark room

March 31, 2014: Week 12

GST lab report due along with your note books