# BIOC23H Winter 2015 Practical Approaches to biochemistry Course outline

Instructor:

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#### Communication

The best way to reach me is, to drop by and see me, or come by during my office hours or at any other time. If I am in my office the door will be open and I will answer your questions at any time. If you wish to see me at a specific time outside of the office hours then email me and request an appointment. I prefer to meet with you to answer your questions. I encourage you to ask questions during lecture. If you have a question about the material, whether it be lecture or laboratory material I encourage you to talk to me during the laboratories as I will be around for much of the lab period or to visit me in my office. It is not feasible to give detailed answers to questions regarding material covered in the lecture or laboratory via email. Therefore I have an open door policy and in addition I hold scheduled office hours

I will answer emails predominately when I am on campus. I am on campus generally Monday through Friday between 9 and 6 pm. If you send me emails on the weekend I will respond no later than following Monday. Please use a utoronto account for email (I will not answer emails from non-U OF T accounts) and please indicate the course in the subject heading.

 General announcements and any material needed for the course will be posted on blackboard

#### Office hours:

Monday 10 am to noon

The objective of this course is to provide the student with the practical experience in biochemical techniques that are used in industry, research and government laboratories. In addition we will provide the background necessary to understand and trouble shoot the techniques used as well as related techniques. Students will gain experience in data analysis and interpretation, with emphasis on scientific writing. Application of biochemical techniques to biological

studies will be a major focus. You will be learning skills that are useful in the job market as well as skills that will be useful in graduate studies.

This is a laboratory course with a 2hr lecture component. The emphasis is on the laboratory component, therefore work in the laboratory and laboratory based material on the midterm and final will represent over 50% of your grade.

## Textbook:

Biochemistry laboratory Modern Theory and Techniques Boyer R 2012 Publisher Pearson, available in bookstore

# **Accessibility:**

Students with diverse learning styles and needs are welcome in this course. In particular, if you have a disability/health consideration that may require accommodations, please feel free to approach me and/or the Access Ability Services Office as soon as possible. I will work with you and Access Ability Services to ensure you can achieve your learning goals in this course. Enquiries are confidential. The UTSC Access Ability Services staff (located in S302) are available by appointment to assess specific needs, provide referrals and arrange appropriate accommodations (416) 287-7560 or ability@utsc.utoronto.ca.

# **Academic integrity/plagiarism**

Academic integrity is essential to the pursuit of learning and scholarship in a university, and to ensuring that a degree from the University of Toronto is a strong signal of each student's individual academic achievement. As a result, the University treats cases of cheating and plagiarism very seriously. The University of Toronto's Code of Behaviour on Academic Matters (<a href="http://www.governingcouncil.utoronto.ca/policies/behaveac.htm">http://www.governingcouncil.utoronto.ca/policies/behaveac.htm</a>) outlines the behaviours that constitute academic dishonesty and the processes for addressing academic offences. Potential offences include, but are not limited to:

(source <a href="http://ctl.utsc.utoronto.ca/home/integrity">http://ctl.utsc.utoronto.ca/home/integrity</a>

## In papers and assignments:

- Using someone else's ideas or words without appropriate acknowledgement.
- Submitting your own work in more than one course without the permission of the instructor.
- Making up sources or facts.
- Obtaining or providing unauthorized assistance on any assignment.

### On tests and exams:

- Using or possessing unauthorized aids
- Looking at someone else's answers during an exam or test.
- Misrepresenting your identity.

## In academic work:

- Falsifying institutional documents or grades.
- Falsifying or altering any documentation required by the University, including (but not limited to) doctor's notes.

All suspected cases of academic dishonesty will be investigated following procedures outlined in the Code of Behaviour on Academic Matters. If you have questions or concerns about what constitutes appropriate academic behaviour or appropriate research and citation methods, you are expected to seek out additional information on academic integrity from myself as your instructor or from other institutional resources

# For the laboratory:

Students require:

- lab coat (no exceptions) and closed toed shoes. You will be asked to leave if you come with inappropriate attire and no lab coat: this will also lead to a loss in associated marks
- 2. safety glasses for most labs
- 3. a permanent black marker (Sanford :sharpie fine point )
- 4. a book for recording your work (your log book). This book can be hard or soft cover, or a binder.

## Safety

- 1. Eating, drinking and use of cell phones 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 <u>Always</u> wear a lab coat (done up) in the laboratory with the sleeves rolled down and closed shoes
- 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 13
- 7. Discard chemical waste in appropriate containers

- 8. Discard bacterial culture material into autoclave bags and wipe benches with alcohol
- 9. Discard pipets <u>point-down</u>, in the upright plastic pipet holders. Make sure the pipet tips are <u>covered</u> with disinfectant.
- 10. Place all test tubes containing living cells in the designated racks/pans;
- 11. discard pipet 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 I chemicals/live cells and also just before leaving the laboratory.
- 13. When leaving the lab, **Wash your hands** and REMOVE YOUR LAB COAT. DO NOT UNDER ANY CIRCUMSTANCES wear your lab coat in any public area.

## Course schedule:

## Lectures:

BIOC23 is a laboratory course. The material covered will relate to the laboratory techniques carried out throughout the course. It will include discussion of methodologies, theory behind the methodology and relevant applications for the particular techniques used. I will also cover the data obtained from the labs carried out. I will address specific aspects of the protocols in each lecture. Lecture gives you a chance to also ask questions regarding the theory behind the labs you will be conducting as well as how these techniques can be applied to various biological questions. Therefore come with questions. Lecture material will be posted by the day of the lecture.

## Laboratories:

- attendance in labs is mandatory. If you miss a lab you must provide a UTSC medical certificate or other acceptable reason cleared by Dr. Brunt to be excused from the lab and to be able to hand in any assignments related to that lab. The is no possibility to makeup labs. Each unexcused missed lab will result in a loss of 4% of your grade. If you miss more than 2 laboratories without proper documentation you will not be given credit for the lab component of the course. If you miss more than 4 labs even if they are excused absences you will be asked to leave the course as this is a laboratory based course.
- Laboratories are posted on blackboard a week before the lab is scheduled to be carried out.
- . It is your responsibility to come prepared each week to carry out your laboratory. To have completed an introduction to each lab (a paragraph) explaining the purpose and to have written out your own flow chart (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 "log it". This means writing down in detail what you did, what you observed, times of incubations. You will have data analysis (including standard curve

construction) **summary / conclusion**, where you analyze the data, do any graphs etc that are required. To ensure you are prepared for the lab there will also be **quizzes** before certain lab topics which will test whether you have adequately prepared for the lab

- The use of a laboratory log book is protocol in any laboratory setting and work learning how to properly document data and experiments is critical and therefore you will be rewarded with grades for doing this
  - 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 at hand

## **Grade Breakdown**

Midterm Monday Feb 23<sup>th 2015</sup> in class

( multiple choice , short answer, short and long essay)

20%

You must contact me within 48 Hours of missing the midterm and provide me with the UTSC medical certificate filled in by your doctor to be able to write midterm. The same medical certificate is required for missed labs

**Final exam** TBA in final exam period( multiple choice , short answer, short essay) (cumulative for laboratory material) 3 hrs 34%

## Date of Quizzes (6 X 0.5%)

3%

Week 1 (differential centrifugation/ mitochondrial isolation (week of Jan 5)

week2 (ion exchange) (week of Jan 12)

week 3: (gel permeation) (week of Jan 19)

week 4-6: (IgG) (week of Jan 26)

week 6-8 (GST/Western) (week of Feb 9)

week 10 (alkaline phosphatase) (week of March 16)

One-minute writes/case studies/reflective writing in lab and lecture (occur randomly, summaries/data presentation in lab/concept maps

must complete a minimum of 80% in lecture and all assignments in lab for full credit)

lecture 3% lab 4%

## Lab performance:

10%

Preparation (intro and flow chart) 2.5% Laboratory log/ summary/data analysis and

Technical performance 7.5%

Your book will be initialed each week and collected at the end. This is a cumulative process if you do not keep you book up to date this will be reflected in

your grade. You may be asked to do small Pass/Fail assignments during class that may be used in the determination of you performance grade

# Lab assignments:

26%

- **1. mitochondrian isolation:** (3.5%) ( maximum 2 page double spaced not including figures, legends, tables, references)
- 2. formal report 1: IgG (8.5%) ( maximum 5 double spaced pages of text not including figures/figure legends/ reference page)
- formal report 2: GST and western (9.5%) (maximum 7 pages of double spaced pages of text not including figures/figure legends/ reference page)
- 4. Alkaline phosphatase, (4.5%) will not require materials and methods requires brief introduction, graphs/with appropriate legends and data analysis ( maximum 1 pages double spaced of text not including graphs, legends or references)
- 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 that is posted.

#### Due dates:

- o Mitochondrial isolation: week of Jan 19th
- o formal report 1: IgG: week o March 2
- o formal report 2: GST and western week of March 23
- o assignment: alkaline phosphatase week of March 30
- formal reports IgG and GST must be submitted electronically TO
   TURNITIN (SEE Below) as well as a paper copy with your name on each
   page and initialed
- If you wish to opt out of turn it in you must inform Dr. Brunt in writing and provide an electronic copy to Dr. Brunt

"Normally, students will be required to submit their course essays to Turnitin.com for a review of textual similarity and detection of possible plagiarism. In doing so, students will allow their essays to be included as source documents in the Turnitin.com reference database, where they will be used solely for the purpose of detecting plagiarism. The terms that apply to the University's use of the Turnitin.com service are described on the Turnitin.com web site".

## Lecture schedule

(I have given topic numbers rather than dates). Lectures relevant to the particular lab will occur either the week before or the week of the laboratory. Some topics will overlap multiple weeks

Topic 1: pH and buffers (will be posted, and you are responsible)

Topic 2: cell fractionation/protein isolation/ purification

Topic 3: chromatography

Topic 4: protein quantification and gel electrophoresis

Topic 5: Immunochemistry/ western analysis

Topic 6: enzyme kinetics

Topic 7: Mass spectrophotometry and information science : use in protein

identification, related protein technologies

Topic 8 Use of isotopes in biochemical studies

## Laboratory schedule for 2015

## Week of Jan 5: Week 1:

<u>Experiment 1:</u> differential centrifugation/ mitochondrial isolation, go over data before you leave the laboratory

Week of Jan 12: week 2

Experiment 2: Ion exchange chromatography

In class reflective practice

Differential centrifugation assignment due

## Week of Jan 19: Week 3:

Finish experiment 2: thin layer chromatography

Experiment 3: gel permeation In class reflective practice

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## Week of Jan 26: Week 4

Experiment 4 part 1: IgG: salting out via ammonium sulfate/dialysis

 write a figure legend for the thin layer chromatography per bench and do group critique and class discussion

# Week of Feb 2: Week 5:

Experiment 4 part 2: IgG: DEAE Sephdex and protein quantification

-finish protein quantification table and determine volume to load on the gel

## Week of Feb 9: Week 6:

Experiment 4 part 3: IgG: SDS gel electrophoresis and staining (picture posted by the following day).

<u>Experiment 5 part 1</u>: Purification of GST fusion proteins from *E.coli* (isolation and affinity chromatography)

# Reading week of Feb 16

Week of Feb 23: Week 7

Experiment 5 part 2: GST fusion protein: gel electrophoresis and Western transfer

Experiment 4: discussion of gel IgG data by each pair: present your gel figure and discuss, one-minute write to summarize the data, go through a example figure legend: do in pairs and critique

## Week of March 2: Week 8

Experiment 5 part 3: GST fusion protein: immunoblot with GST antibody/dark room, data returned via blackboard

IgG report due

Week of March 9: Week 9

Experiment 5: group powerpoint presentation: full analysis of GST presented,

Week of March 16: Week 10

Experiment 6: enzyme kinetics: alkaline phosphatase

Week of March 23: Week 11

Data analysis of enzyme kinetics, in class presentation of data

GST lab report due

Week of March 30: Week 12

No lab: Alkaline phosphatase assignment due along with your note books at a time to be arranged with your TA today