

BIOC23H3: Practical Approaches to Biochemistry

Course Outline

Summer 2021

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Labs will be a combination of on-line synchronous and in person:

In person laboratories will start Thursday June 10. Please see the lab schedule for details. The first four weeks will be on-line synchronous

Lecture will be synchronous on-line from 9 to 11 am

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. The course will expand your knowledge of more advanced techniques. Students will gain experience in experimental design, data analysis and interpretation, with emphasis on scientific writing, oral presentation and critical thinking. 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 in person laboratory course with a 2-hour lecture component which will be a synchronous virtual meeting. The emphasis is on the laboratory component therefore the final exam will be based on application of biochemical techniques.

During the synchronous lecture **we will do a number of exercises that will apply the material covered in lecture that is applicable to laboratory exercises. You therefore must come prepared for lecture. Read the e-text and the lecture notes before class.** **A number of lectures will involve the use of extensive break out groups with students solving problems to help improve understanding.** The lecture notes provided are detailed enough for a standalone review and the text provides excellent background and problems to solve.

Learning outcomes:

- Understand the role of biochemical techniques in protein study
- Evaluate the function of an enzyme using appropriate controls
- Compare and contrast types of chromatography, gel electrophoresis and protein purification and protein/protein interaction methods and evaluate the

- advantages of each
- Compare and contrast experimental design and develop appropriate experimentation to ask specific questions
 - Formulate hypotheses and develop strategies to investigate them.
 - Create effective data collection protocols
 - Explain the importance of reproducible data and demonstrate an understanding of the limitation of that data
 - Create research objectives through thoughtful design using existing literature
 - Evaluate data collected and present the data in an appropriate manner in both an oral, or written presentation.

Communication

I will hold virtual office hours following lecture. I will also be available in person by appointment during the in-person laboratory sessions. If you need to speak to me privately we can arrange a video chat.

I will answer email between 9 and 5 pm Monday through Friday. **Please use U of T account for email and please do not use the email function in Quercus. (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 Quercus.

Office hours: available for 30 mins immediately after lecture

Textbook you can use (recommendation):

Biochemistry laboratory Modern Theory and Techniques Boyer R 2012 Publisher Pearson, available in bookstore. The text is strongly recommended. It is available as an e-book through Pearson publishing. <https://www.pearson.com/store/p/biochemistry-laboratory-modern-theory-and-techniques/P100000230132/9780321830579>

This book is an excellent resource for theory and provides many practice questions and I highly recommend you purchase this text. If you take any other advanced lab in molecular biology, it will also be valuable. It should also be available through the [bookstore](#)

Equity Statement:

- The University of Toronto is committed to equity, human rights and respect for diversity. All members of the learning environment in this course should strive to create an atmosphere of mutual respect where all members of our community can express

themselves, engage with each other, and respect one another's differences. U of T does not condone discrimination or harassment against any persons or communities.

AccessAbility:

AccessAbility statement

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 AccessAbility Services Office as soon as possible.

AccessAbility Services staff (located in Rm AA142, Arts and Administration Building) are available by appointment to assess specific needs, provide referrals and arrange appropriate accommodations 416-287-7560 or email ability@utsc.utoronto.ca. The sooner you let us know your needs the quicker we can assist you in achieving your learning goals in this course.

Academic integrity/plagiarism

The University treats cases of cheating and plagiarism very seriously. The University of Toronto's Code of Behaviour on Academic Matters (<http://www.governingcouncil.utoronto.ca/policies/behaveac.htm>) outlines the behaviours that constitute academic dishonesty and the processes for addressing academic offences.

Potential offences in papers and assignments include 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 cheating includes using or possessing unauthorized aids, looking at someone else's answers during an exam or test, misrepresenting your identity, or falsifying or altering any documentation required by the University, including (but not limited to) doctor's notes. <http://www.utsc.utoronto.ca/vpdean/academic-integrity>

Examples of plagiarism

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 your instructor or from other institutional resources. <https://www.utsc.utoronto.ca/vpdean/faq-0>

Use of Turnitin (plagiarism software) in BIOC17

All assignments and your final take home exam will be deposited to Turnitin via Quercus submission

"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".

Intellectual Property

Recording or photographing or video capture of any aspect of a university course - lecture, tutorial, seminar, lab, studio, practice session, field trip etc. – without prior approval of all involved and with written approval from the instructor is not permitted.

INSTRUCTOR PERMITS AUDIO RECORDINGS WITH NO DISTRIBUTION RIGHTS

Students may create audio-recordings of the lectures for their personal use. Recordings are intended to permit lecture content review so as to enhance understanding of the topics presented. Audio-recordings are not a substitute for attending class.

Students should note that since audio recordings are to be permitted, their voice may be recorded by others during the class. Please speak to the instructor if this is a concern for you.

In accordance with the Accessibility for Ontarians with Disabilities Act, 2005, persons who have special needs will be accommodated.

Students agree to the following terms when creating audio recordings of lectures:

- Recordings are not to be distributed without the permission of the instructor via the Internet, using social media such as Facebook, peer-to-peer file sharing such as One Drive or Dropbox, or other distribution channels.
- Recordings are not to be shared with other classmates unless they are to be used in collaborative assignments, or if the instructor permits for other reasons.

Grade breakdown and self-declaration for work that cannot be completed:

- **For lecture and laboratory assignments that cannot be handed in on time:** late assignments will have a 10% per day deduction- no assignment will be accepted more than 5 days late. **The weekly Pre- and Post-lab questions cannot be submitted late.**

If you are ill you must submit biology specific self-declaration within 24 hrs of the due date

- All assignments are submitted electronically to Quercus

Self-declaration for illness:

- If you are self- declaring you must fill out the self-declaration form and submit it to myself and Jennifer Campbell within 24 hrs of the assignment due date. **See department web site** <https://www.utsc.utoronto.ca/biosci/node/389>

For the laboratory (your laboratory is a level one Biosafety laboratory): In the era of COVID you will be given more detailed Guidelines but below are general guidelines for all laboratories and are absolutely required- if you arrive for lab without your PPE or permanent marker you will not be permitted entry.

Students require:

1. **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** or **plexiglass face shield** for all labs which must remain on throughout the lab.
3. **a permanent black marker** (Sanford:sharpie fine point)- we will not supply markers
4. **a book for recording your work** (your log book). This book can be hard or soft cover, or a binder. For your own use only.
5. **A bag to place your lab coat in** - used to bring your coat to lab and place your coat in after laboratory completion.

In the laboratory General Rules (COVID RULES UNDER SEPARATE COVER)

1. **Eating, drinking, chewing gum 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. You may not touch or handle in any way your cell phone
2. **NO food or drink can be left outside of the laboratory**
3. **Please do not bring** coats, hats, etc. into the laboratory if at all possible.
4. **You must Always wear a lab coat** (done up) in the laboratory with the sleeves rolled down and **closed shoes.**
5. Wear gloves and safety glasses for the entire laboratory.
6. Keep paper, pencils, fingers, etc. out of your mouth. Do not share writing implements
7. At the beginning and end of the laboratory, follow procedures outlined for you in detail in a separate document.
8. Discard chemical waste in appropriate containers.

9. Discard bacterial culture material into autoclave bags and wipe benches with alcohol.
10. Discard pipets point-down, in the upright plastic pipet holders. Make sure the pipet tips are covered with disinfectant.
11. Place all *test tubes* containing living cells in the designated racks/pans;
12. Discard pipet tips in the appropriately marked container.
13. **Wash hands thoroughly with soap and water on entry and exit from the lab.**
14. 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 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 prior to the lecture.

Laboratories:

- **attendance in labs is mandatory. If you miss a lab, you must provide a self-declaration or other acceptable reason cleared by Dr. Brunt. Self-declaration Documentation must be submitted to Dr. Brunt and Jennifer Campbell 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 unexcused missed lab will result in a loss of 5% of your grade and will prohibit handing in lab work.
- **You can self-declare for two laboratories only.**
- **If you miss 3 laboratories (regardless of the reason you will not be given credit for the lab component of the course (and you will not pass the course and will be asked to leave the course as this is a laboratory-based course. If you are seriously ill or diagnosed with COVID. I will address those individually so contact me immediately on diagnosis.**
- **Laboratories are posted on Quercus at least 4 days before the lab is scheduled to be carried out.**

It is your responsibility to come prepared each week to carry out your laboratory. This includes: 1) Complete and submit to Quercus your pre-lab assignment (intro. & flow chart) for each lab module. 2) Have a copy of the flow chart in your lab notebook and keep complete notes in this book. 3) Complete and submit the weekly post-lab assignment.

When carrying out experiments in person 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. This will be used to write up your weekly laboratory assignments and lab reports. The use of a laboratory log book is protocol in any laboratory setting (work or research) and **learning how to properly document data and experiments is critical and therefore you will be rewarded with grades for doing this. We will not be grading the lab books due to COVID but they will be needed for your success in your assignments and for success in the comprehensive final take home exam. The PRE-LAB and POST LAB replaces the lab note book.**

Grade Breakdown

Final take home exam which will be a cumulative essay exam based on all of the lecture and laboratory material TBA in final exam period.	33%
Research proposal: Due August 10- I have given you the entire term to work on this (introduced in week one). There will be no extensions for this assignment- see Quercus. This assignment includes the following: <ul style="list-style-type: none"> • Concept Map – due June 21st 1.5% • Annotated Bibliography – due July 14th 1.5% • Research proposal final paper – due Aug 10th 13% 	16%
Pre-lab assignments (introductory paragraph & flow charts) for 6 laboratory modules – see Quercus	6%
Lab performance (includes technical performance, oral presentations, breakout group/class discussions)	8%
Small weekly laboratory assignments due after the laboratory for a number of lab sessions-percentages will change with the particular week – see Quercus	7%
One-minute writes/case studies/reflective writing in lecture (occur randomly, must complete a minimum of 80% in lecture) you may opt out and have it moved to the final exam	4.5%
Laboratory Major Assignments:	25.5 %
1. Alkaline phosphatase (5%); this will not require materials and methods; it requires brief introduction, graphs with appropriate legends and data analysis (maximum 1 page discussion double-spaced of text not including graphs, legends, or references. Please follow the outline posted to you in week one.	
2. mitochondrial isolation: (3.5%) (maximum 2-page double spaced not including	

- figures, legends, tables, references)
- 3. formal report 1: IgG (7.5%)** (maximum 5 double spaced pages of text not including figures/figure legends/ reference page)
 - 4. formal report 2: GST and western (9.5%)** (maximum 7 pages of double-spaced pages of text not including figures/figure legends/ reference 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 that is posted.

Due dates for major laboratory assignments (ALL due at 10 pm electronically):

- Alkaline phosphatase assignment due on May 27th see Quercus
- Mitochondrial isolation report: Due June 3rd see Quercus
- Formal report 1 – IgG: Due Jul 17th see Quercus
- Formal report 2 – GST: Due August 3rd see Quercus

Lecture schedule for 2021

Material will be posted a week in advance. Much of the lecture material will be done using examples, therefore I will not cover the material in detail for all lectures. You are responsible for preparing for the lecture so we can try to use example questions to address the topics. (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. All lab procedures will be reviewed in the lecture.

Topic 1: pH and buffers (will be posted, and you are responsible for the material, I will not cover in class)

Topic 2: enzyme kinetics

Topic 3: cell fractionation/protein isolation/ purification

Topic 4: chromatography

Topic 4: protein quantification and gel electrophoresis

Topic 6: Immunochemistry/ western analysis

Topic 7: Mass spectrophotometry and information science: use in protein identification, related protein technologies

Topic 8: Use of isotopes and new technologies in non-radioactive monitoring in biochemical studies

Laboratory schedule for 2021

- All **pre-lab assignments** include an introductory paragraph and flowchart outlining the procedure for each lab module - due before your scheduled lab time.
- All **post-lab assignments** – due 1 week after lab unless otherwise indicated on Quercus (this replaces the data analysis, standard curves, answers to critical thinking questions that are usually in your lab notebook)

Week 1 May 13: On-line synchronous module

Pre-lab assignment – Expt. 6 - Alkaline Phosphatase Lab module

During lab: Introduction to research proposal; Explanation and Posting of Alkaline Phosphatase data

Post-lab assignment - NONE

Week 2 May 20: On-line synchronous module

Pre-lab assignment – Expt. 1- Partial Purification of mitochondria using differential centrifugation Lab module

During lab: Class discussion of concepts and data during lab

Post-lab assignment – One paragraph summary – data analysis

Week 3 May 27: On-line synchronous module

Pre-lab assignment – Expt. 2- Ion exchange chromatography Lab module

During lab: Class discussion of concepts and data

Post-lab assignment – Figure legend of chromatogram & critical thinking question (See Quercus – Assignments for details)

Reminder: Alkaline phosphatase report due May 27th at 10 pm

Week 4 June 3: On-line synchronous module

Pre-lab assignment – Expt. 3 – Gel Permeation Lab Module AND

Read Expt. 4 – IgG partial purification from Rabbit Serum (you do not need to prepare flowchart for IgG)

During Lab: Class discussion of concepts and data for Gel Permeation; Introduction to Experiment 4 - part 1 IgG – Salting out via ammonium sulfate/dialysis

Post-lab assignment - One paragraph summary of results for Gel Permeation; answers to critical thinking questions (see Quercus – Assignments for details)

Reminder: Mitochondrial Isolation report due June 3rd at 10 pm

Week 5 June 10: IN-PERSON

Pre-lab assignment – Expt. 4 - IgG partial purification from Rabbit Serum (intro and flowchart for all 3 days of procedure)

During Lab: Experiment 4 part 2: IgG - DEAE Sephadex, protein A and protein quantification

Post-lab assignment – Standard Curve; Complete protein quantification table and determine volume of samples to load on gel next week. (see Quercus – Assignments for details)

Week 6 June 17: IN-PERSON

Pre-lab assignment – Expt. 5 - Affinity Purification of a Glutathione-S-transferase fusion protein (intro and flowchart for all 3 days of procedure)

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

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

Post-lab assignment – Prepare gel figure for your IgG presentation in week 7 and

write a figure legend. This will be submitted to Quercus and you will receive feedback before your presentation.

Reminder: Concept Map – due June 21st at 10pm

June 24 (reading week) and July 1 (holiday)- no classes

Week 7 July 8: IN-PERSON

Pre-lab assignment – NONE

During lab: Experiment 5 part 2: GST fusion protein: gel electrophoresis and Western transfer; Experiment 4: discussion of gel IgG data by each pair -

Please send the PowerPoint Slide by email to your TA prior to lab so your TA can put the files on the computer. You will present per pair standing on the opposite side of the screen. Then a classroom discussion on the figure legend submitted.

Post-lab assignment – Write a one-paragraph summary of Ponceau S stained membrane and Coomassie Blue Stained Transfer gel results (maximum 1/2 page); Include pictures of each

Reminder: Annotated Bibliography – due July 14th at 10pm

Week 8 July 15: IN-PERSON

Pre-lab assignment – NONE

During Lab - Experiment 5 part 3: GST fusion protein: immunoblot with GST antibody/ development-CNS, data returned via Quercus; review GST presentation format/requirements.

Post-lab assignment – Standard Curve for unstained markers; Use this to determine size of pre-stained markers; Standard Curve for pre-stained markers and determination of size of fusion protein; Brief summary of Uninduced vs. Induced Gel.

Reminder: Formal report 1 – IgG: Due Jul 17th at 10pm

Week 9 July 22: On-line synchronous module

No Pre- or Post-lab Assignments

During Lab - Group PowerPoint presentation: full analysis of GST presented.

Complete class discussion and break out groups. By the end of this lab, you will have your figures labeled and major concepts developed for your lab report due next week.

Week 10 July 29: IN-PERSON

No Pre lab

In lab: gel permeation lab that was discussed virtually in week 4 to demonstrate how to run a column. We will follow EXPT.3 discussed in week 4.

Post lab: summary of V_o , V_e and V_T

Week 11 August 5: NO LAB

Reminder: Research proposal final paper – due Aug 10th at 10pm_Reminder no extensions for any reason.