

BIOCHEMISTRY LABORATORY - CHEM 567

Prerequisites: Chemistry 560 or equivalent.
Strongly recommended: 562/564 series as a corequisite or prerequisite.

Rooms: All laboratory work and lectures for the course (all sections) will normally commence in CSL226. We will not normally be using CSL332, but it will be used on occasion, please listen for announcements. If you are late and the class is not where you thought it would be, maybe it is in the other room.

Course description/topics: This is a combined lecture and laboratory class. The lectures will cover what you need to know to successfully complete the labs, but additional material is often covered to expand your knowledge of modern biotechnology. We will cover isolation of membranes, separation of membrane components, enzyme kinetics, protein purification and analysis, DNA isolation, PCR, recombinant DNA, and associated topics such as tissue culture, sequencing, and use of radioactivity. You will work with a group to complete all tasks so communication and planning is essential.

Instructors: The laboratory will be taught by Kathleen McNamara Schroeder.
Office: CSL 313 Telephone: 594-1614 kmcnamara@mail.sdsu.edu
Office hours: I am around the 3rd floor most of the day, either office. come whenever you have a question. The hour just before class is usually bad, because I will be trying to get everything ready for lab.

Please get Computer Room magnetic card access request slips from the Chem office during the first week of classes. Pick up your magnetically-encoded cards by presenting this slip along with your SDSU ID to the key issue office (Public Safety). This card will permit you to enter the computer lab, GMCS 234.

Grading for Biochemistry Laboratory (Chem 567) – Fall '14

		<u>Total</u>
1. Four Exams (one at the end of each module)	150 points each	600
2. Photosystems and LDH Kinetics Lab Reports	100 points each	200
3. Aldolase Lab Report	150 points	150
4. Plasmid, PCR/STR, and GST-SH2 Lab Reports	75 points each	225
5. DNA sequencing homework	25 points	25
6. Satisfactory Lab Notebook	100 points	<u>100</u>
	Total Points:	1300 points

The following grades are guaranteed for the points shown. It is possible that the points may be lowered, but they will not be raised for a given letter grade.

A	1170-1300 points	D	780-909 points
B	1040-1169 points	F	779 and below
C	910-1039 points		

Required course material:

- a. Chem 567 "Biochemistry Laboratory" by Stumph , Metzger and Adams in loose-leaf folder in Aztec Shops bookstore. This is shelved under the name "Stumph". The 2008-9 version includes material excerpted from Methods in Enzymology. Do not use older versions.
- b. A bound laboratory notebook with carbon copies

You may wish to wear a lab coat during the class, it will prevent stained clothing which is certainly possible during some of the experiments. It may be kept in the locker if desired.

A bound laboratory notebook with carbon copies is required. Notes taken during your labwork **must** be entered directly into this notebook, and not merely taken down on loose paper. Therefore, you must **always** bring your notebook to class. It is also perfectly acceptable to take laboratory lecture notes in this notebook. Prior to the end of the semester, your notebook will be examined **without advance warning** by the TA. It will be graded satisfactory or unsatisfactory. The grading criteria is based upon the completeness of your notebook. If judged satisfactory, you will receive 100 points. If your notebook is unsatisfactory, you will not receive these points. If you do not have your notebook with you for collection by the TA, there will be an immediate loss of 50 points! **You should always have your notebook with you for this laboratory class.** Notebooks will not be accepted if they are more than one class period late.

There will not be a final exam.

There will be 4 major sections or modules. There will be a 50 minute exam following each of these modules given during the Monday lecture period. If you are late for an exam, no extra time will be allowed.

Lab reports. Each experiment will require its own lab report that will be due approximately 1 week after completing the experiment. (See schedule for details.) There will be 6 lab reports required [Photosystems; LDH Kinetics; Aldolase; Plasmid Prep; PCR/STR; GST-SH2 Expression and Purification].

There may be days when this class runs over the scheduled lab times. Usually it is just a short time, but on the day when we run the phosphocellulose column in the Aldolase lab it will be significant (1+ hours over depending upon your preparation for the experiment). Choose your lab groups carefully to ensure that at least one person in the group is able to stay until the experiment is finished. I do not recommend taking a class which begins at 5 pm on Tuesday/Thursday.

There will be an introductory and explanatory lecture given before or at the beginning of each experiment. This will usually occur in the Monday lecture period. (In some cases, lectures may be given during the lab periods, or given over several periods)

Lab Reports:

Organize each lab report as follows:

A. Introduction. One single space typed page minimum. Usually takes two for a full explanation. The Introduction will contain the following:

- 1) Brief background of the topic of research
- 2) Purpose of the experiment
- 3) General plan to accomplish the experiment
- 4) Theory behind the lab techniques mentioned in (3).

Typically, 1, 2, and 3 occupy about 1/2 to 1 page, and 4 occupies a full page.

B. Flow chart. One page, unless otherwise noted.

This should be a general outline of the experimental procedures to be followed.

Important notes:

1. Sections A and B (i.e., Introduction and Flow Chart) should be written as if you were planning out this experiment in your own mind prior to initiating work on the experiment.
2. Sections A and B must also be included in your lab notebook. (It is acceptable to type A and B, copy them, and then tape these copies into your notebook. You may also write out A and B in your notebook and hand in the detachable carbon copy.)
3. Sections A and B must be handed in to the TA at the **beginning** of the lab period on the day they are due. This is usually, but not always, the day on which you begin the experiment. See schedule for due dates.
4. Sections A and B will then be graded and will normally be handed back before your final lab report is due. You will then staple these graded sections onto the rest of your completed lab report prior to handing it back to the TA.

C. Experimental Procedures: If you are provided with a detailed step-by-step protocol, you only need to mention any actual variations from the protocol provided. However, be sure to mention any and all deviations. You do not have to re-copy the protocols from the laboratory manuals. If there is not a detailed protocol provided for a particular experiment, you will need to include more details in this section. This section will be handwritten in your notebook, but should be typed for your lab report.

D. Results: The length of this section will vary from experiment to experiment. Present the results in the most clear and understandable fashion possible. This may involve Figures, Tables, Charts, and/or Graphs, **but must also contain typed explanatory text**. You cannot just present a collection of tables and graphs and call that your Results section; **you must explain in words** what the charts and graphs demonstrate.

E. Conclusion and Discussion: One page typewritten maximum. Discuss the results of your experiment. In your introduction there was a purpose statement. Address this statement by explaining the results gathered, and if the experiment accomplished what was said in the purpose. Finally, do include error analysis. Describe what errors may have taken place **and the impact that these errors had on the experiment**.

Students with disabilities- If you are a student with a disability and believe you will need accommodations for this class, it is your responsibility to contact Student Disability Services at [\(619\) 594-6473](tel:6195946473). To avoid any delay in the receipt of your accommodations, you should contact Student Disability Services as soon as possible. Please note that accommodations are not retroactive, and that accommodations based upon disability cannot be provided until you have presented your instructor with an accommodation letter from Student Disability Services.

Learning Objectives

By the end of this semester students will be able to:

1. Understand the purpose of each chemical component in an experiment, and how it aids in the ultimate goal of the experiment.
2. Troubleshoot an experiment with the intention of improving the outcome.
3. Operate standard biochemistry laboratory equipment including centrifuges, thermocyclers, gel apparatus and power supplies, spectrophotometer, shakers, fraction collectors, and gravity chromatography columns.
4. Perform techniques common to biochemistry lab including PCR, electrophoresis, western blots, kinetics, expression of recombinant proteins, isolation and purification of DNA, and growth of bacterial cells.
5. Write results of experiments in lab reports that include background information on the topic, a plan to accomplish the experiment, an outline of the theory behind relevant lab techniques, and results/discussion of the experimental outcome.

Your Lab Notebook:

1. Leave several pages blank at the beginning of the notebook to keep an up-to-date Table of Contents.
2. Introduction and Flow-chart: To be written prior to starting the experiment. Copies are to be handed into the TA on the date due, but copies must also be kept permanently in the notebook, placed there on the date each intro is due.
3. Experimental Procedures and Results: Record any and all deviations from the protocols provided in the lab manuals or handouts, whether these are intentional or done in error. Record primary data (obtained during the lab period) and derived data. That is, record your raw data as it is gathered, and then show how you get from your raw data to your final tabulated data.
4. Conclusion and Discussion: Same as for lab report, this is also placed in the notebook the day it is due in class.
5. Your entries into the lab notebook do not have to be pretty. However, one should be able to read and follow what was written. The goal of keeping a notebook is to be able to take a primary protocol along with your research notebook and be able to completely **understand and reproduce** the work that was accomplished.

Notice from the above instructions that **your lab report and your lab notebook will contain much overlapping material**. But this is not to add to your work, but to save you work, as most pages can be Xerox copies! For example, the Introduction, Flow-chart, and Conclusion and Discussion should be identical in your lab notebook and in the lab report you hand in. However, the Experimental Procedures and much of what is considered Results will be spontaneously entered into your notebook throughout the lab. As a result, the Experimental Procedures and the Results will often be intermingled in your notebook. Therefore, **these two sections (Experimental Procedures and Results)** should be **re-organized** and typed for the **lab report** that you hand in.

Important: Please note that I take a strict stand with regard to handing in material late. I take the due dates very seriously!

My policy:

Failure to hand in the **Introduction and Flow Chart** at the **beginning of class** on the date due: **10 point penalty**. (I expect you to come to class prepared.) This penalty will be subtracted from the total points. Moreover, if you do not hand in a copy when initially

due, you are still expected to prepare and include these sections as part of your lab report, or you will **lose even more points** in the grading of your report.

Lab reports are due at the **beginning of class** on the specified date. **Late lab reports will be accepted up to the first class period following the date due, but will be subjected to a 10 point penalty.** Thus, a perfect but late lab will automatically be penalized 10 points.

Finally, even though you will be working in groups, I expect everyone to be in class and to participate in doing the experiment. If you miss an experiment without the instructor's permission, you will **not** be allowed to hand in a lab report. **No free rides!**

FLOW-CHART EXAMPLE

Large Scale Plasmid Preparation - Flow Chart

Day 1

1. Prepare and autoclave media.

Day 2

1. Inoculate 1 liter cell cultures. Incubate with shaking at 37°C.
2. Read absorbance of culture at 2, 2.5, 3 hrs etc.
3. When A_{600} reaches 0.55-0.6, add chloramphenicol. Incubate overnight and centrifuge cells on the following morning.

Day 3

1. Thaw cell pellet and lyse cells on ice.
2. Remove cell debris by centrifugation in Sorvall high speed centrifuge. Save supernatant containing the plasmid DNA.
3. Set up CsCl-ethidium bromide equilibrium gradient centrifugation. Spin in ultracentrifuge at 41,000 rpm in TV-850 rotor at least 18 hours.

Day 4

1. Bring down ultracentrifugation run. While illuminating with UV light, remove plasmid DNA band from centrifuge tube using needle and syringe.
2. Set up second CsCl-ethidium bromide centrifugation in TV650 rotor. Spin at 41,000 rpm at least 18 hours.

Day 5

1. etc.

*Sometimes, depending upon the particular experiment, it may be preferable to **diagram** the flow chart as shown on the following page Try to choose whichever method (or combination of the two) is best for getting across the points most clearly.*

