

Chemistry 493
Biochemistry Laboratory

Spring 2009

M 2:00 - 2:50 PM (lecture)
T 8:00 - 11:50 AM
T 1:00 - 4:50 PM
Th 8:00 - 11:50 AM

Professor: Dirk Iwata-Reuyl (iwatareuyl@pdx.edu)
506 SBI x-5737
Office hours: **M** 3:00-4:00, **W** 1:00-2:00, or by appointment.

Teaching Assistants: Tuesday morning: Nilesh Vaidya (vaidyn@pdx.edu)
Tuesday afternoon/Thursday morning: Chris Deutsch (cdeutsch@pdx.edu)

Text: Biochemistry Laboratory: Modern Theory and Techniques, by Rodney Boyer, 2006, Benjamin Cummings.

Required Items: Laboratory Notebook with carbon or duplicate sheets
Calculator
Safety Goggles/Glasses

Prerequisites: Ch 320/321, Ch 490/491.

Course Overview: Chemistry 493/593 is a one-term course in experimental biochemistry, and serves as an introduction to experimental laboratory techniques common to biochemical research laboratories, with an emphasis on protein biochemistry. The structure of the laboratory is project based, with all of the separate weekly labs comprising steps in the purification and characterization of the enzyme lactate dehydrogenase (LDH). Each laboratory period lasts 3 hours and 50 minutes, so it will be essential to manage your time efficiently. The time to plan and prepare for an experiment is *before* you enter the laboratory!

Grading: The final grade will be based on 5 parts: **1)** written lab reports (50% of the grade), **2)** the lab notebook and pre-labs (10%), **3)** homework (10%), **4)** weekly quizzes (10%), and **5)** cheerfully and responsibly carrying out the labs (20%).

- Lab Report and pre-reports: There will be 1 comprehensive written report on the lactate dehydrogenase project (20%) and 3 pre-reports (30%). These must be written in the style and format of a manuscript submitted to the Journal of Biological Chemistry (JBC). Specific instructions can be found at the Journal web site (<http://www.jbc.org/misc/ifora.dtl>); *reports must be prepared and submitted in accordance with these instructions*. Note that manuscripts submitted to JBC that do not follow these instructions are returned without review; for the purposes of this course reports submitted that do not conform to the Journal instructions will be returned for rewriting or reformatting, and if late will be docked points (see below). All reports must be *fully referenced* (*i.e.* check your results with the literature and appropriately cite these references) and free of grammatical and spelling errors. All graphs should be prepared with data analysis software (*e.g.*, GraphFit, KalidaGraph, PS Plot, Excel, etc.). If are inexperienced in constructing graphs see the Excel tutorial on the LabWrite website (<http://www.ncsu.edu/labwrite/res/res-homepage.htm>). For the general content of a lab report see the textbook and the LabWrite site. For examples of appropriate scientific

writing browse recent issues of JBC or other relevant journals. I *strongly* encourage those with weak writing skills to set up an appointment *now* at the writing center (Cramer Hall Rm 188F, <http://www.writingcenter.pdx.edu/>). Due dates for reports are listed in the syllabus. Late reports will be docked 5% per day (of the total possible score).

- **Notebooks:** Laboratory notebooks are permanent (**in ink**) records of the sequence of events that occur during an experiment. Entries should be an accurate description of the procedures followed and the phenomena observed. They should be sufficiently detailed so that a researcher who is experienced, but not familiar with the particular techniques being described, can reproduce the experiment **exactly**. The entries in a laboratory notebook should be so inclusive that writing up an experimental procedure, including necessary references, data, etc., can be prepared without resorting to any outside material. Notebook grades will be issued at the beginning and end of each lab period. The duplicate sheets *must* be turned in at the conclusion of each laboratory period to receive notebook credit (it is *your* responsibility to turn these in). The initial grade will be based on general preparation for the lab as deduced from an organized outline of the days experiment(s), relevant calculations for the preparation of reagents and buffers, and a list of specific pre-lab questions and any other questions you may have about the experimental protocol (you may have many or none). The pre-lab questions are a set of general questions relevant to *any* experiment that you might do. See the LabWrite site (<http://www.ncsu.edu/labwrite/pl/pl-homepage.htm>) for these general questions (in most cases you will be doing descriptive labs). The second grade, assigned at the end of each laboratory period, will be based on the *organized and legible* entry of the raw data and the actual protocol followed, with relevant observations or comments.
- **Homework:** Assignments will cover specific elements of each laboratory and are designed to get you thinking about these issues, as well as introducing you to some of the relevant calculations, before you carry begin the lab. Homework will be due at the beginning of the Monday afternoon class period. *Late homework will not be accepted* (we typically discuss the homework problems in class).
- **Laboratory Quizzes:** A quiz will be given each week during the Monday afternoon lecture session. Quizzes will cover assigned reading material from both the text and the laboratory handouts. No make-up quizzes will be allowed, but the lowest grade will be dropped.
- **Cheerfully and responsibly carrying out the labs:** This recognizes the considerable effort that must be put into a lab course. Grading will be based on your general level of preparedness for the labs, your willingness to carry your share of the work-load and not make your partner do everything, whether you work safely or are a menace to everyone in the lab, etc.

Syllabus

Week	Reading	Lab Activities
March 30	Ch 1, 2, 3A	Introduction, lab check-in, buffer preparation
April 6	Ch 4, 11A Ch 6 (sec. 1) in V&V Handout	Isolation and Characterization of Avian Lactate Dehydrogenase: <i>Preparation of cell-free extract</i>

April 13	Ch 3B, 5A, C-G Ch 6 (sec. 3) in V&V	Isolation and Characterization of Avian Lactate Dehydrogenase: <i>Affinity chromatography, Bradford analysis</i>
April 20	Ch 7A, 8B	Isolation and Characterization of Avian Lactate Dehydrogenase: <i>Fraction activity assays</i> Pre-report #1 due
April 27	Ch 14 (sec. 2 & 3) in V&V	Isolation and Characterization of Avian Lactate Dehydrogenase: <i>Kinetics of lactate formation, inhibition kinetics</i>
May 4		Isolation and Characterization of Avian Lactate Dehydrogenase: <i>Kinetics of pyruvate formation</i> Pre-report #2 due
May 11	Ch 5E Ch 6 (sec. 4) in V&V	Isolation and Characterization of Avian Lactate Dehydrogenase: <i>Native molecular weight determination by gel filtration</i> Pre-report #3 due
May 18	Ch 6A, B	Isolation and Characterization of Avian Lactate Dehydrogenase: <i>SDS-PAGE analysis</i>
May 25	Ch 6C	Isolation and Characterization of Avian Lactate Dehydrogenase: <i>Western blot analysis</i>
June 1		Lab Make-up and Check-out <i>Final lab report due Monday, June 9</i>

Required contents of pre-reports

- Preliminary lab report #1: Methods and Results for i) CFE preparation, ii) affinity chromatography, iii) Bradford analysis.
- Preliminary lab report #2: Methods and Results of fraction assays for LDH activity (complete purification table)
- Preliminary lab report #3: Introduction
Methods and Results kinetic experiments