

Biomolecular Chemistry 504  
HUMAN BIOCHEMISTRY LABORATORY  
Spring 2008

**LABORATORY SCHEDULE**

*Lab Period: 1-9 Dr. Denu; 10-17 Dr. Fillingame; and 18-27 Dr. Fox*

| <b><u>Period</u></b> | <b><u>Date</u></b> | <b><u>Title/Agenda</u></b>   |
|----------------------|--------------------|--|
| 01                   | 1/22               | <u>Introduction to lab techniques</u><br>1) Introduction and safety talk.<br>2) General announcements.<br>3) Short lecture on spectrophotometry and $A = \epsilon c l$ .<br>4) Demonstration of spectrophotometers, pipettor and other equipment.<br>5) Pipetting and Diluting Techniques laboratory.  |
| 02                   | 1/24               | <u>Protein Purification</u> – spectrophotometry and purification methods<br>1) Lecture: Protein spectral properties and Protein purification.<br>2) Demonstration of gel permeation chromatography.<br>3) Demonstration of dialysis.<br>4) Dialysis of a serum sample for ion exchange chromatography. |
| 03                   | 1/29               | <u>Protein Purification</u> - ion exchange chromatography<br>1) Ion-exchange Chromatography for the Purification of IgG.<br>2) Other serum protein fraction saved for affinity chromatography.<br>3) IgG fraction saved for SDS-polyacrylamide gel electrophoresis                                     |
| 04                   | 1/31               | <u>Protein Purification</u> - affinity chromatography<br>1) Affinity Chromatography for the Purification of Fibronectin.<br>2) Dialysis of purified fibronectin for enzyme immunoassay.  |
| 05                   | 2/05               | <u>Proteins</u> - Blood proteins<br>1) Units & Spectrophotometry mini-quiz (20 points).<br>2) Lecture: Blood proteins<br>3) Protein Absorption Spectrum, Hematocrit and Plasma Preparation.  |
| 06                   | 2/07               | <u>Enzyme Kinetics</u><br>1) Enzyme Kinetics: Alkaline Phosphatase Part A and B  |
| 07                   | 2/12               | <u>Enzyme Kinetics</u><br>1) Enzyme Kinetics: Alkaline Phosphatase Part C and D  |
| 08                   | 2/14               | <u>Enzyme Kinetics</u><br>1) Enzyme Kinetics: Alkaline Phosphatase Part E  |
| <b><u>Period</u></b> | <b><u>Date</u></b> | <b><u>Title/Agenda</u></b>   |
| 09                   | 2/19               | <u>Blood Proteins/Enzymes</u> - clotting factors<br>1) Lecture: Blood clotting.<br>2) Blood Clotting Experiments.  |
| --                   | 2/21               | <b>Lab Exam 1 (covering lab periods 1-9)</b>   |

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| 10                   | 2/26               | <u>Coupled Enzyme Assays</u><br>1) Lecture: Coupled Enzyme Assays<br>2) Aspartate Aminotransferase (AST) Assay   |
| 11                   | 2/28               | 1) Lecture: Introduction to Metabolic Adaptation   |
| 12                   | 3/04               | <u>Metabolic Adaptation</u><br>1) Normal rat.  |
| 13                   | 3/06               | <u>Metabolic Adaptation</u><br>1) Fasted rat.<br>2) High carbohydrate diet rat.  |
| 14                   | 3/11               | <u>Metabolic Adaptation</u><br>1) Diabetic rat.<br>2) Diabetic rat + insulin.  |
| 15                   | 3/13               | <u>Metabolic Adaptation</u><br>1) Unknown rat  |
|                      | 3/15-3/23          | <i>Spring Recess</i>   |
| 16                   | 3/25               | <u>Metabolic Adaptation</u><br>1) Unknown rat, Complete or repeat assays and checkout.<br>2) Prepare with group members for the presentation of unknown.<br>3) Optional Discussion: Metabolic Adaptation (by lab instructors).<br>4) Metabolic Adaptation Questions. |
| 17                   | 3/27               | <u>Metabolic Adaptation</u><br>1) Group Presentation and Discussion of the Metabolic Adaptation Laboratory<br>2) Receive DNA/Metabolic Protein Report  |
| --                   | 4/01               | <b>Lab Exam II (covering lab periods 10-17)</b>  |
| <b><u>Period</u></b> | <b><u>Date</u></b> | <b><u>Title/Agenda</u></b>   |
| 18                   | 4/03               | <u>Protein Analysis</u> - Serum proteins<br>1) All: Serum Protein Analysis: Electrophoresis and Total Protein Determination.   |
| 19                   | 4/08               | <u>Protein Analysis</u> - EIA I<br>1) Lecture: Enzyme Immunoassay (EIA).<br>2) Fibronectin EIA. (Incubate plate with fibronectin.)   |
| 20                   | 4/10               | <u>Protein Analysis</u> - EIA II<br>1) Fibronectin EIA (conjugate and substrate incubation).<br>2) Lecture: SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western Blot during EIA conjugate incubation   |

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| 21                   | 4/15               | <u>Protein Analysis</u> –SDS-PAGE & Western Blot<br>1) Western Blot: SDS-PAGE (3 person groups)<br>2) Lecture: SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western Blot continued during the electrophoresis<br>3) Western Blot: Transfer to Nitrocellulose<br>4) SDS-PAGE: Fill out worksheet needed for SDS-PAGE sample preparation for the next lab period.<br>5) EIA Problem Set and lab report due. |
| 22                   | 4/17               | <u>Protein Analysis</u> -SDS-PAGE & Western Blot<br>1) SDS-PAGE: Protein analysis by SDS Polyacrylamide Gel Electrophoresis (2 person groups)<br>2) Western Blot: Incubation with antibodies, etc.   |
| 23                   | 4/22               | <u>DNA Techniques</u><br>1) Human Genetic Typing: Part A. - DNA prep & Part B- PCR<br>2) Lecture: Human Genetic Typing<br>3) Receive Human Genetic Typing computer/database problem set.<br>4) Human Genetic Typing: C. - Restriction enzyme digests   |
| 24                   | 4/24               | <u>DNA Techniques</u><br>1) Human Genetic Typing: Part D. - Agarose gel electrophoresis<br>2) Lecture: Intro to Genome sequencing and databases. Begin Recombinant DNA Techniques<br>3) Recombinant DNA Techniques: Day 1. - DNA ligation<br>4) View agarose gel with your group.  |
| <b><u>Period</u></b> | <b><u>Date</u></b> | <b><u>Title/Agenda</u></b>   |
| 25                   | 4/29               | <u>DNA Techniques</u><br>1) Lecture: Continued Recombinant DNA Techniques<br>2) Recombinant DNA Techniques: Day 2a. - DNA transformation; Day 2b. – Concentration of competent cells determination.<br>3) Complete Human Genetics Typing lab report.<br>4) Complete Human Genetic Typing computer/database problem set.<br>5) Present DNA/Metabolic Protein Report- 2 Groups-only if necessary for those groups    |
| 26                   | 5/01               | <u>DNA Techniques and Protein Analysis</u><br>1) Recombinant DNA Techniques: Days 3a, 3b. - Count colonies.<br>2) Complete Recombinant DNA Techniques lab report.<br>3) Present DNA/Metabolic Protein Report- 6 Groups   |
| 27                   | 5/06               | 1) Exam Review and Discussion<br>2) Present DNA/Metabolic Protein Report- 6 Groups   |
| --                   | 5/08               | <b>Lab Exam III (covering lab periods 18-27)</b>   |

