Protein Bioseparation Methods
Course Outcome Summary

Course Information

Organization	MATC - Madison Area Technical College
Developers	Lisa Seidman
Development Date	2/13/2003
Course Number	10-007-122
Potential Hours of Instruction	126

Description
Introduces the general strategies commonly used to purify proteins. Specific methods include determining specific activities for enzymes, extraction of proteins from bacterial cells, salting out, dialysis, ion exchange chromatography and polyacrylamide gel electrophoresis.

Types of Instruction

Instruction Type	Contact Hours	Credits
Classroom Presentation	18	
On-Campus Laboratory and Clinicals	108	
Individualize/Independent Study		
Simulated or Actual Work Experience		
On-the-Job Experience		

Textbooks
Springer-Verlay. *Protein Purification: Principles and Practice*. **Edition:** 2nd. **ISBN:** 3-540-96555-6.

Biotechnology Staff. *Resource package*.

Biotechnology Staff. *Lab manual*.

Learner Supplies
Long-sleeved lab coat.

Calculator.

Safety glasses.

Prerequisites
007-104 or consent of instructor.
006-112 or consent of instructor.

Competencies

Unit I. Overview of Protein Separations Methodology

1. Identify and explain commercial applications of protein purification methodology.

You will demonstrate your competence:
- o through the completion of written assignments.

Your performance will be successful when:
- o you find examples of commercial protein separations in articles.

- you explain the significance of the products.

2. Outline a strategy for a protein purification from memory.

You will demonstrate your competence:

- through written examinations.

Your performance will be successful when:

- your strategy includes extraction method, low resolution technique, high resolution technique and assay.
- your strategy is consistent with guidelines in text and references.

3. Identify and explain physical and chemical properties of proteins.

You will demonstrate your competence:

- through the completion of written assignments.
- through written examinations.

Your performance will be successful when:

- you relate the physical and chemical properties of a protein to separation methods.

4. Design a separation strategy based on application of knowledge in 1.3 above.

You will demonstrate your competence:

- through the completion of written assignments.
- through written examinations.

Your performance will be successful when:

- you are able to use simulated data from hypothetical protein to design separation strategy.

Unit II. Extraction

1. Explain protein extraction from plant, animal and bacterial cells.

You will demonstrate your competence:

- through the completion of written assignments.
- through written examinations.

Your performance will be successful when:

- your methods are explained according to references.

2. Write a flowchart outlining the extraction of beta galactosidase from E. coli.

You will demonstrate your competence:

- through the completion of written assignments.
- through written examinations.

Your performance will be successful when:

- your flow chart includes all steps in lab manual and indicates where samples are removed, volumes determined and assays performed.

3. Grow and collect E. coli cells on shaker.

You will demonstrate your competence:

- through the completion of laboratory assignments.
- through the completion of written documentation and explanation of laboratory work.
- through the completion of written explanations of laboratory data.
- through completion of an oral report to class.

Your performance will be successful when:

- with a pellet of at least 0.5 grams.

4. Assay supernatant for protein.

You will demonstrate your competence:

- through the completion of laboratory assignments.
- through the completion of written documentation and explanation of laboratory work.
- through the completion of written explanations of laboratory data.
- through completion of an oral report to class.

Your performance will be successful when:

- o straight line on standard curve; assay result falling on standard curve from sample.

5. Assay supernatant for beta galactosidase activity.

You will demonstrate your competence:

- o through the completion of laboratory assignments.
- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Your performance will be successful when:

- o by discernible yellow color in sample tube within 10 minutes.

6. Calculate specific activity of supernatant.

You will demonstrate your competence:

- o through the completion of written assignments.
- o through written examinations.
- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Your performance will be successful when:

- o your calculations are correct.
- o your results are between 1,000 and 20,000 units/mg.

7. Keep accurate records of findings.

You will demonstrate your competence:

- o through the completion of laboratory assignments.
- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Unit III. Precipitation of Proteins

1. Describe strategy to precipitate protein.

You will demonstrate your competence:

- o through the completion of written assignments.
- o through written examinations.

Your performance will be successful when:

- o your strategy is consistent with textbook guidelines.

2. Precipitate beta galactosidase from supernatant above.

You will demonstrate your competence:

- o through the completion of laboratory assignments.
- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Your performance will be successful when:

- o your activity in redissolved pellet using assays learned in competency 2.4.

Unit IV. Desalting Proteins and Changing Buffers

1. Describe how to desalt a sample and change buffers.

You will demonstrate your competence:

- o through the completion of written assignments.
- o through written examinations.

Your performance will be successful when:

- o your description includes all steps of desalting.

2. Perform dialysis.

You will demonstrate your competence:

- o through the completion of laboratory assignments.
- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Your performance will be successful when:

- o you dialysate with pH of proper buffer, proper conductivity and activity as assayed using methods learned in competency 2.4.

Unit V. Ion Exchange Chromatography

1. Explain principles of chromatographic separation.

You will demonstrate your competence:

- o through the completion of written assignments.
- o through written examinations.

Your performance will be successful when:

- o your explanation is correct according to checklist.

2. Explain cationic and anionic exchangers.

You will demonstrate your competence:

- o through the completion of written assignments.
- o through written examinations.

Your performance will be successful when:

- o your explanation is correct and distinguishes between types of exchangers.
- o your explanation discusses pH and charge.

3. Set up chromatography column with DEAE gel.

You will demonstrate your competence:

- o through the completion of laboratory assignments.
- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Your performance will be successful when:

- o according to manual instructions, all parts are in correct orientation; no bubbles in connecting tube. (Diagram and instructions are in lab manual and Pharmacia booklet.)

4. Run sample on chromatography column.

You will demonstrate your competence:

- o through the completion of laboratory assignments.
- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Your performance will be successful when:

- o collected fractions.

5. Graph data from column fractions.

You will demonstrate your competence:

- o through the completion of laboratory assignments.
- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Your performance will be successful when:

- o your graph is similar to that instructor obtained using the assays learned in competency 2.4.

Unit VI. Concentration of Samples

1. Using centricon apparatus, concentrate sample pooled from column fractions.

You will demonstrate your competence:

- o through the completion of written assignments.
- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Your performance will be successful when:

- o you produce concentrated sample.

Unit VII. Electrophoresis of Protein Samples

1. Write a short explanation of theory of polyacrylamide gel electrophoresis and isoelectric focusing.

You will demonstrate your competence:

- o through the completion of written assignments.
- o through written examinations.

Your performance will be successful when:

- o you explain differences between the two methods.

2. Cast a PAGE gel.

You will demonstrate your competence:

- o through the completion of laboratory assignments.
- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Your performance will be successful when:

- o your gel is completed.

3. Run samples on PAGE.

You will demonstrate your competence:

- o through the completion of laboratory assignments.
- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Your performance will be successful when:

- o your gel is completed.
- o mw markers with correct spacing.
- o effective protein separation.

4. Perform a Western Blot.

You will demonstrate your competence:

- o through the completion of laboratory assignments.
- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Your performance will be successful when:

- o you perform blot and B galactosidase band is present.

5. Cast an Isoelectric Focusing (IEF) Gel.

You will demonstrate your competence:

- o through the completion of laboratory assignments.
- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.

- o through completion of an oral report to class.

Your performance will be successful when:

- o your IEF gel is cast.

6. Transfer IEF gel to SDS PAGE gel and run samples.

You will demonstrate your competence:

- o through the completion of laboratory assignments.
- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Your performance will be successful when:

- o you demonstrate completed two-dimensional gel.

Unit VIII. High Performance Liquid Chromatography

1. Explain purpose and operation of HPLC.

You will demonstrate your competence:

- o through the completion of written assignments.
- o through written examinations.

Your performance will be successful when:

- o you give correct explanation according to checklist.

2. Run samples on HPLC.

You will demonstrate your competence:

- o through the completion of laboratory assignments.
- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Your performance will be successful when:

- o your mw markers are separated and identified.
- o you separation of proteins results in clear peaks.

Unit IX. Record Keeping and Interpreting Results

1. Maintain records of laboratory procedures and array results.

You will demonstrate your competence:

- o through the completion of laboratory assignments.
- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Your performance will be successful when:

- o you maintain records for entire unit according to checklist.

2. Compile and organize results from purification project.

You will demonstrate your competence:

- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Your performance will be successful when:

- o all aspects are included.
- o your data is organized in tabular form.
- o your graphs and gels are present and labeled.
- o your calculations are correct.

3. Present data from purification project.

You will demonstrate your competence:

- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Your performance will be successful when:

- o your presentation is clearly organized as in 9.2.
- o your calculations are shown in stepwise form.

4. Discuss purification project in a critical fashion.

You will demonstrate your competence:

- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Your performance will be successful when:

- o you participated with comments during lab presentations in competency 8.2.
- o you interpret calculations correctly.
- o you interpret graphs and gels correctly.
- o you point out inconsistencies and questions in data.

5. Communicate project results in written report.

You will demonstrate your competence:

- o through the completion of laboratory assignments.
- o through the completion of written documentation and explanation of laboratory work.
- o through the completion of written explanations of laboratory data.
- o through completion of an oral report to class.

Your performance will be successful when:

- o your specific activity is summarized and explained for the entire project. Problems and poor results are discussed. Suggestions for changes in future work given.
- o all criteria in 9.2, 9.3 and 9.4 are met in writing.