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Laboratory Experiment

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# Purification and Electrophoretic Characterization of Lactate Dehydrogenase from Mammalian Blood: A Different Twist on a Classic Experiment

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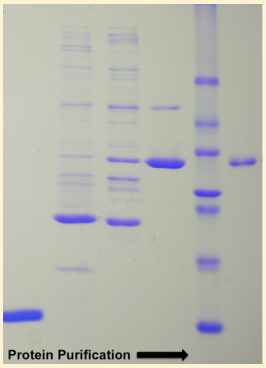
Supporting Information

**ABSTRACT:** A multiweek protein purification suite, suitable for upper-division biochemistry or biotechnology undergraduate students, is described. Students work in small teams to isolate the enzyme lactate dehydrogenase (LDH) from a nontraditional tissue source, mammalian blood, using a sequence of three column chromatographic procedures: ion-exchange, size exclusion, and affinity chromatography. Protein and enzyme activity elution profiles are determined by graphical analysis of assay data collected using rapid microplate spectrophotometric assays. Students perform quantitative assays on LDH pools and use these data to build a purification table for use in evaluating the protocol. The protocol typically generates final overall fold-purifications from 1500 to 2500 and activity recoveries of 45−60%. Electrophoretic separations in both denaturing and native-gel format are analyzed both visually and by use of commercial digital analysis software to assess the isoenzyme pattern of isolated LDH and to further evaluate the purification. Assessment of student work revealed a high level of achievement of course learning goals that include development of critical thinking skills required to (1) critically evaluate an experimental protocol and (2) draw conclusions based on a variety of forms of experimental data.




**KEYWORDS:** *Upper-Division Undergraduate, Biochemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Bioanalytical Chemistry, Chromatography, Electrophoresis, Enzymes, Ion Exchange*

## INTRODUCTION

The development of a biochemistry laboratory course that introduces students to classical and modern biochemical techniques often involves overcoming a number of challenges, one being limited resources. Chief among the resources that are often in short supply are (1) funds for the purchase of materials and disposal of waste, (2) technical support to prepare large quantities of needed reagents, and (3) modern instrumentation adequate to allow students to complete the various tasks in a reasonable amount of time.

A second challenge is to design a suite of laboratory experiences that allows for a multiweek project that provides, when possible, protocols mirroring the materials and methods found in the primary literature rather than those found in a more cookbook style of laboratory manual. This fits well with the goal of the upper division laboratory course to provide a window into the world of research, requiring students to pursue tasks such as protocol optimization and timely analysis of the large amounts of data that such endeavors produce. This goal is of critical importance both to students who will eventually progress to graduate school and to students who will enter into industrial positions upon graduation.

A third challenge is that of providing an environment that stimulates and supports cooperative learning among the individuals in a student laboratory team, encouraging students to have joint responsibility for the success or failure of their projects.

In redesigning our Biochemical Techniques course, we sought to develop a project that would embrace these challenges. We chose the enzyme lactate dehydrogenase (LDH) as the target molecule for isolation and electrophoretic characterization. While previous papers[1−6] and laboratory manuals[7,8] have described the isolation of this protein from mammalian heart or skeletal muscle, we chose to do the isolation from mammalian blood, a nontraditional source that offers a number of advantages. (1) Red blood cells (RBC) are easily isolated in pure form from whole blood by repeated centrifugation in isotonic saline; (2) the cytosolic fraction, or membrane-free lysate, is generated by simple hypotonic lysis followed by centrifugation; (3) the resulting lysate yields LDH activity that is stable for several weeks, allowing a measure of flexibility with regard to the timing of its preparation for the class; (4) the lack of any significant protease activity in the lysate alleviates the need to add expensive and toxic protease inhibitors; (5) the fold purification and percentage recovery of activity are far superior to those typically reported for the isolation of LDH using mammalian muscle tissues.[2−4]

The purification protocol utilizes a set of three column chromatographic procedures that have found wide application in biochemistry: ion-exchange, size exclusion, and affinity

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chromatography. Rapid microscale analysis of protein concentration and LDH activity in column fractions, as well as the starting material and the three chromatographic purification "pools", is accomplished by use of a microplate reader and accompanying processing software. Electrophoretic behavior is investigated by native polyacrylamide gel electrophoresis (PAGE), denaturing SDS−polyacrylamide gel electrophoresis (SDS−PAGE), and Western blot analysis using visual examination as well as computer-assisted densitometric analysis. Students summarize their results in a formal written journal style report, orally defend a poster presentation of their work, or prepare a series of progress reports, one after each stage of the purification and a short summary analysis at the end of the multiweek project. The second half of the term students utilize the wide-range of skills they have developed by embarking on independent or small group research-based projects optimizing protocols from the literature.

Learning goals for this project are listed in Box 1. The current study describes the progress made in the development of the experimental protocol and provides an assessment of student success in attaining specific learning goals and objectives.

**Box 1. Learning Goals**

Goal 1: Acquire an understanding of the general features of a variety of common chromatography resins and understand how intermolecular forces influence the elution behavior of proteins during separation via column chromatography

Goal 2: Develop proficiency in carrying out microscale analytical procedures and in utilizing appropriate software to do necessary data analysis

Goal 3: Gain experience in carrying out a variety of commonly encountered electrophoretic separations and in utilizing appropriate software to carry out necessary data analysis

Goal 3: Demonstrate the importance of deductive reasoning in using various pieces of analytical data to assess the efficacy of a purification protocol and to evaluate the quality of data generated by an experimental protocol

Goal 5: Gain experience in handling biochemical reagents in a safe manner

## EXPERIMENTAL METHODS SUMMARY

A detailed description of the experiment is provided in the Supporting Information. Students worked in groups of 2−4 to complete this experiment over the course of 6 weeks, meeting for 4 h laboratory periods twice a week. A brief synopsis of the order in which the tasks are performed is given below.

### Materials and Software

Rabbit blood was purchased from Hemostat Laboratories (Dixon, CA). Anti-LDH-H-subunit monoclonal primary antibody, chromatography resins, LDH standards, and electrophoresis reagents were purchased from Sigma-Aldrich (St. Louis, MO). Precision Plus prestained molecular weight standards for electrophoresis were obtained from Bio-Rad laboratories (Hercules, CA). Goat-anti-mouse-alkaline-phosphatase secondary antibody was purchased from Rockland Immunochemicals (Limerick, PA). All other reagents were reagent grade. UN-SCAN-IT-gel software (Silk Scientific, Orem, UT) was used to perform densitometric analysis on stained electrophoresis gels. SoftMax Pro software (Molecular Devices, Sunnyvale, CA) was used to analyze microplate assay data.

### Synopsis of the Experimental Suite

**First Week.** Students begin the purification by carrying out ion-exchange column chromatography. They obtain a sample of membrane-free lysate prepared by hypotonic lysis of washed rabbit RBC (Stage 1) and apply it to a column of Carboxymethyl-Sephadex (CM-Sephadex). The column is developed overnight at 4 °C by isocratic elution in phosphate buffer. The following day students perform spectrophotometric assays to assess elution profiles for protein and LDH activity. Protein elution is determined by mixing small aliquots of each column fraction with Bradford protein assay reagent in a 96-well plate. This reagent contains Coomassie Brilliant Blue G-250, a dye that exhibits an absorbance shift under acidic conditions to a blue form upon binding to proteins.[9] The increase in absorbance at 595 nm is proportional to the concentration of protein in the sample and may be readily determined in a microplate reader. The relative concentration of LDH activity in each column fraction is determined by mixing a small aliquot of the fraction with an assay "cocktail" containing phosphate buffer, sodium pyruvate, and NADH in a 96 well plate and then measuring the rate of decrease in absorbance at 340 nm, associated with oxidation of NADH,[10] in a microplate reader. Data are presented as the $V_{max}$ or initial rate as determined by the use of SoftMax Pro analysis software. The data are used to prepare a graphical representation of the relative elution of protein (absorbance at 595 nm) and LDH activity ($V_{max}$); students consult with the instructor to determine which column fractions to combine to maximize recovery of LDH. The dilute combined pool is concentrated using centrifugal ultrafiltration to prepare the final CM-LDH pool (stage 2).

**Second Week.** Students continue the purification by applying 75% of their CM-LDH pool onto a Sephacryl S-200 gel filtration column, developing the column overnight at 4 °C in phosphate buffer supplemented with ammonium sulfate. The following day, they perform relative protein concentration[9] and LDH activity[10] microplate assays on column fractions as noted above and prepare a graphical representation of the data. They then consult with the instructor to determine which fractions to combine to maximize the recovery of enzyme activity and minimize contamination from closely eluting proteins. The desired fractions are combined to prepare the S-200-LDH pool (stage 3).

**Third Week.** Students continue the purification by applying 75% of their S-200-LDH pool onto a Cibacron Blue affinity chromatography column, using NADH-containing phosphate buffer to effect affinity elution from the resin. They perform relative protein concentration[9] and LDH activity[10] microplate assays on column fractions as noted above, prepare a graphical representation of the data the same day and combine fractions containing LDH. The dilute pool is then concentrated using centrifugal ultrafiltration to prepare the Cibacron-LDH pool (stage 4).

**Fourth Week.** Students perform quantitative LDH activity microplate assays[10] to determine the activity in units per milliliter (units/mL) for each pool. $V_{max}$ data, as well as information regarding the volume of each pool used, dilution factors, well path length, and the millimolar extinction coefficient of NADH at 340 nm, are used to determine the concentration of LDH activity in each pool. In this experiment, a "unit" of LDH activity


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is defined as the amount required to convert 1 $\mu$mol of NADH to $NAD^+$ per minute at room temperature (20–22 °C).

Students also carry out microscale Bradford[9] protein assays on each pool and prepare bovine serum albumin and LDH standard curves. They then determine the final protein concentration in each pool, in milligrams of total protein per milliliter (mg total protein/mL), by comparison to the most appropriate standard curve (albumin for Stages 1–3; LDH for Stage 4). The data are summarized in a purification table, listing the LDH activity (units/mL), mg total protein/mL, and total volume for each pool. This information is used to determine the specific activity (units/mg total protein), the overall fold purification (specific activity of a pool/specific activity of lysate starting material), and overall percentage recovery of LDH activity.

**Fifth Week.** Students perform SDS–PAGE[11] on molecular weight standards, an LDH standard, and samples from each pool. After the gel is stained, they take digital photos of the stained gel and use UN-SCAN-IT-gel software (Silk Scientific, Orem, UT) to analyze the Coomassie blue staining patterns to calculate the molecular weight of rabbit RBC LDH and estimate the relative purity of LDH in each pool. They perform native PAGE[12] on bovine heart LDH, rabbit skeletal muscle LDH, and samples from each pool, and use an LDH activity stain to evaluate the isoenzyme pattern in each sample.

**Sixth Week.** If time permits, students may perform an optional Western blot[13,14] analysis of bovine heart LDH standard and one or more of the LDH pools using monoclonal antibodies raised against the H-subunit of LDH. Each student group prepares final written and/or oral reports summarizing the results of the multiweek experiment, using the Internet to find needed references for standards such as the molecular weight of mammalian LDH, isoelectric points of proteins, etc. At the conclusion of the experiment, students work with the instructor or TA to dispose of or properly store all student-generated materials. Student summary reports compare a variety of data streams (spectrophotometric and electrophoretic analyses) to make conclusions to evaluate the success of the purification and make suggestions regarding possible improvements or extensions to the protocol.

## HAZARDS

Additional information on safety and hazards is included in the detailed protocol in the Supporting Information. All work should be done wearing appropriate splash goggles, appropriate gloves, and lab coat. Toxic, volatile, and/or flammable reagents such as 2-mercaptoethanol, methanol, and isopropyl alcohol should be used in a fume hood. Glacial acetic acid is corrosive and flammable and Bradford reagent is a corrosive liquid. Acrylamide and sodium azide are highly toxic. Many of the other reagents are toxic and/or irritants; instructors should familiarize themselves with the MSDS information readily available for each compound prior to the start of the experiment and consult with appropriate authorities regarding waste disposal.

## RESULTS AND DISCUSSION

After each stage of the purification, students perform assays on all column fractions to determine the relative elution position of protein and LDH activity; these data are used to guide the choice of column fractions to combine to form the partially purified LDH pools (Figure 1). At the conclusion of the ion-exchange separation, hemoglobin, which has a higher isoelectric point than does LDH and is present in high concentration in RBC lysate, remains almost exclusively bound to the top of the CM-Sephadex column. This affords students a potent visual confirmation of the influence of isoelectric pH on elution behavior.

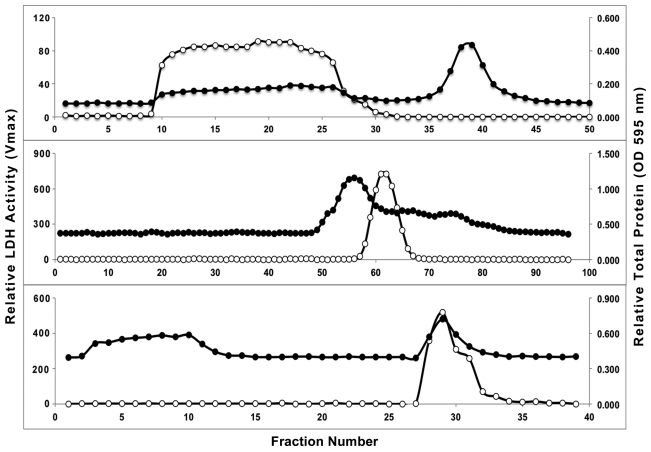


**Figure 1.** Column chromatographic purification of LDH from rabbit RBC lysate. LDH was purified from lysate via three successive column chromatographic protocols as noted in Experimental Methods Summary. Column fractions were assayed to determine the relative LDH activity (open circles) by measuring the initial rate of decrease in absorbance at 340 nm ("$V_{max}$"). Column fractions were assayed to determine the relative protein concentration using a Bradford[9] microplate protein assay (closed circles), measuring the absorbance at 595 nm. Upper panel: ion-exchange chromatography; fractions 10–26 were combined and concentrated by centrifugal ultrafiltration to prepare the CM-LDH pool. Middle panel: subsequent size exclusion chromatography; fractions 60–64 were combined to prepare the S-200-LDH pool. Lower panel: subsequent affinity chromatography; fractions 1–10: sample adsorption; fractions 11–26: buffer wash; fractions 27–39: NADH/buffer elution; fractions 28–33 were combined and concentrated by centrifugal ultrafiltration to prepare the Cibacron-LDH pool.

Following completion of the purification suite, students perform quantitative protein assays and LDH enzyme activity assays on the four stages of the purification. The results are used to determine the specific activity of LDH, the overall fold purification, and the overall recovery of LDH activity after each stage of the purification. The data for a typical student team is summarized in Table 1. Typical final fold purification values range from 1500 to 2500, with final overall recoveries after affinity chromatography from 45 to 60%, indicating a significantly more successful isolation, relative to data published for isolation of LDH from mammalian muscle.[2−4]

Aliquots of student-generated LDH purification pools and LDH standards are next subjected to three different forms of electrophoretic analysis (Figure 2) as described in the Experimental Methods Summary. Densitometric analysis of staining patterns is used to assess the relative purity of LDH at each stage of the purification and to estimate the subunit molecular weight by reference to migration of molecular weight standards; students later compare their experimental values to those they are able to find in the primary literature.

### Student Achievement of Learning Goals

One hundred forty-four students during the past 8 years have completed the basic experimental protocol in 11 offerings of our upper-division Biochemical Techniques course. Students taking the course are typically upper-division undergraduate biochemistry majors; prior to enrolling in the course, they all complete a lower division biology laboratory course that includes


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**Table 1. Purification Table Summary of Representative Student Data for LDH Purification from RBC Lysate**

| Purification Stage (Pool Name) | Volume of Pool (mL) | Total Protein Concentration (mg total protein per mL)[a] | LDH activity (units per mL)[a,b] | Specific Activity (units of LDH Activity per mg total protein)[c] | Overall Fold Purification of LDH[c,d] | Overall Percentage Recovery of Units of LDH Activity (Relative to Lysate)[c,e] |
|---|---|---|---|---|---|---|
| 1. Membrane-free lysate | 130 ± 1[f] | 27.9 ± 0.9 | 1.10 ± 0.05 | 0.040 ± 0.002 | N/A | N/A |
| 2. Ion-Exchange Chromatography (CM-LDH) | 4.00 ± 0.05 | 5.53 ± 0.09 | 33.4 ± 0.9 | 6.0 ± 0.2 | 153 ± 10 | 93 ± 5 |
| 3. Size Exclusion Chromatography (S-200-LDH) | 9.00 ± 0.05 | 0.236 ± 0.007 | 10.0 ± 0.4 | 43 ± 2 | 1080 ± 60 | 84 ± 5 |
| 4. Affinity Chromatography (Cibacron-LDH) | 1.75 ± 0.05 | 0.293 ± 0.010 | 23.7 ± 0.5 | 81 ± 3 | 2050 ± 130 | 52 ± 2 |

[a]Error is given as standard deviation of quadruplicate determinations; the relative standard deviation is typically 1−10% for activity and protein assays. Students have the option to repeat an assay if the relative error is outside of this range. [b]One unit of LDH activity is defined as the amount of enzyme required to convert 1 $\mu$mol of NADH to $NAD^+$ per minute. Beer's law is used to determine the change in NADH concentration using a millimolar extinction coefficient of 6.22 $mM^{-1}$ $cm^{-1}$ and a microplate well path length of 0.75 cm. [c]Error is calculated as the absolute error.[15] [d]Overall fold purification is calculated as the ratio of the specific activity of the pool divided by the specific activity of the lysate starting material. [e]Recovery calculations take into account that students only applied 75% of CM-LDH and S-200-LDH pools to the next column in the series. [f]130 mL of lysate was prepared from 10 mL of packed RBC (approximately 50 mL of whole blood).

some exposure to the use of micropipettes, spectrophotometric assays, and graphical analysis of data. Their skills in these areas range from novice to good at the beginning of the Biochemical Techniques course. Student progress in polishing these skills is monitored informally by allowing students to consult with the instructor or TA for quick feedback as they collect spectroscopic data and by having students submit draft versions of graphical analyses for critique prior to submitting final versions for grading. This form of low stakes informal assessment typically indicates a solid improvement in these skill areas for all students in the course. During the term, students make excellent progress in attaining proficiency in the use of tools such as micropipettes, although some struggle with liquid transfers of the small volumes ($\leq$10 $\mu$L) often required for the spectrophotometric assays. In the instances where students make significant errors in microplate assays and request to repeat the assays, the new analyses are consistently carried out correctly. Students quickly master the use of microplate reader kinetics software to determine enzyme activity, as well as the use of spreadsheets to prepare high quality graphical analyses of their data sets.

In addition to classroom observation, analyses of student laboratory reports and written exams are used to assess student achievement of course learning goals (Table 2). Written exams include questions keyed to these goals. In many cases, the questions are short answer or multiple-choice questions directly related to specific operations students are to perform in lab that week or had performed the previous week. For example, to evaluate Learning Goal 1, students are asked to briefly explain the role of specific reagents in the lab protocols (e.g., the role of NADH in the purification of LDH using Cibacron blue affinity resin or the role of SDS in gel electrophoresis) or to briefly explain the specific protein property (size, charge, etc.) being exploited to effect separations in each kind of chromatography employed during the term. To evaluate Learning Goal 4, students are given a scenario that details the step-by-step protocol an investigator followed to carry out an enzyme assay. The scenario contains 8 separate errors (incorrect temperatures, incorrect volumes, incorrect use of micropipettes, incorrect use of plate reader kinetics software, etc.). Students are asked to describe the errors and to suggest how to modify the protocol to secure better data. Another question provides a different scenario in which an investigator purified a target enzyme using a series of chromatographic procedures similar to those used in the course. Students are asked to explain how the effectiveness of the purification could be evaluated (a) by doing comparisons of enzyme specific activity at each stage of the purification, and (b) by doing visual and/or computer assisted comparison of Coomassie blue staining patterns of SDS−PAGE gels lanes of the different protein pools. Yet another question presents students with a group of proteins of differing molar masses and asks them to predict the order of elution from a gel filtration column and to predict how specific changes in the elution protocol (changing pH, temperature, elution speed, column dimensions) might impact the quality of the separation. Learning Goal 2 is assessed primarily by review of student generated graphs and data presented in the summary purification table, comparing student data to data generated by the instructor's analysis of student purification pools. The data presented in Table 2 indicate that, while there was a wide range of abilities in the assessment group, on average, these students had a high level of achievement of the course learning goals.

Narrative evaluations completed at the end of the term allowed students to comment on how successful the multiweek experiment was at helping them to achieve a variety of learning objectives such as gaining experience in (1) performing microscale bioanalytical procedures, (2) the prompt processing of large data sets and preparing graphical representations of data, (3) using a variety of data to draw conclusions about the success of a multiweek experimental protocol, (4) learning how to use a variety of kinds of software to prepare and analyze digital images, and (5) working effectively in lab teams. Numerical student ratings indicate a high degree of satisfaction with the experiment (3.9−4.6 on a scale of 1−5). Students commented on how "intense" the course was, with one student saying the experiment was "bootcamp for how to be a scientist". Another student indicated that they liked the wide range of skills they were exposed to and that they got to see the entire process of an experiment "doing it, analyzing it, presenting it".

## ■ CONCLUSIONS

The experimental suite developed meets each of the three challenges we sought to overcome. With regard to the "resources" challenge, the use of a microplate reader allows for rapid analyses[16] and immediate assessment of data quality, permitting students the option to reassay samples when encountering problematic data points. This instrument uses much less reagent



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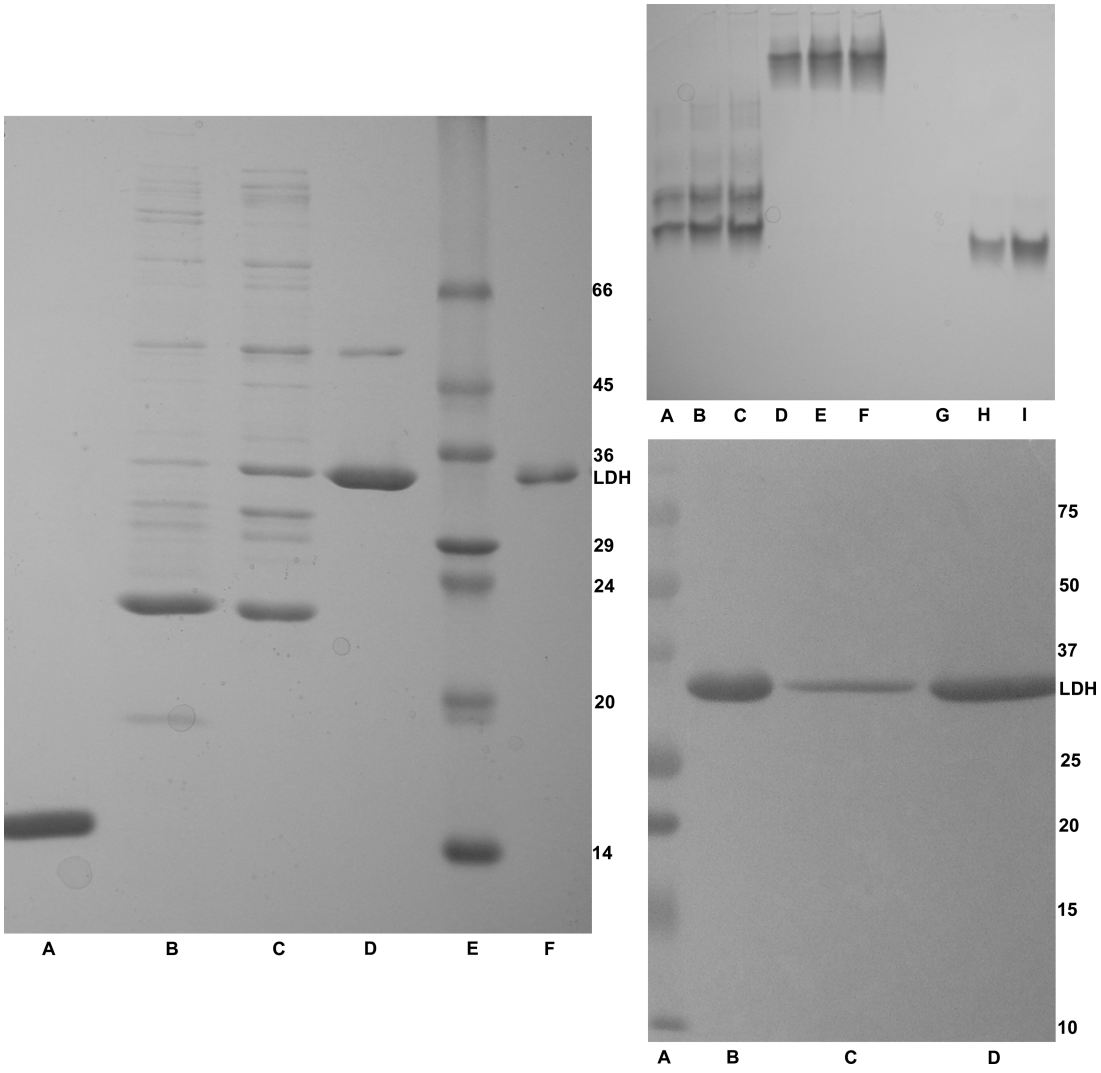


**Figure 2.** Electrophoretic analysis of standard and student LDH samples. Left panel: SDS−PAGE analysis; lane A, lysate pool (Stage 1; 15 $\mu$g total protein); lane B, CM-LDH (Stage 2; 15 $\mu$g total protein); lane C, S-200-LDH (Stage 3; 15 $\mu$g total protein); lane D, Cibacron-LDH (Stage 4; 15 $\mu$g total protein); lane E, molecular weight standards; lane F, LDH standard (10 $\mu$g). The molecular weight in kilodalton (kDa) for the standards and the migration position for the LDH standard are noted to the right of the gel photo. UN-SCAN-IT-gel analysis of the Coomassie blue staining pattern in lane D indicated that the major band, corresponding to the migration of LDH, was approximately 92% of the total protein in the sample. Upper right panel: LDH activity stained native gel analysis; lanes A−C, bovine heart LDH (2.5, 5, 10 $\mu$g, respectively); lanes D−F, rabbit skeletal muscle LDH (2.5, 5, 10 $\mu$g, respectively); lane G− I, 5 $\mu$g of CM-LDH, S-200-LDH, and Cibacron-LDH, respectively. Lower right panel: Western blot analysis; lane A, prestained molecular weight standards; lane B, bovine heart LDH (15 $\mu$g); lane C, S-200-LDH (Stage 3; 20 $\mu$g total protein); lane D, Cibacron-LDH (Stage 4; 20 $\mu$g total protein). The molecular weight in kDa for the standards and position of LDH are noted to the right of the blot photo.

than traditional spectrophotometers using standard cuvettes, thus reducing cost and preparation time. A single plate reader may be used to allow up to 4 student teams to run numerous assays in a single 4-h class period, eliminating the need for multiple standard spectrophotometers in the classroom.

With regard to the "cooperative learning challenge", over the past eight years numerous student teams have displayed the ability to share responsibility for the lab work, data analysis, and report preparation associated with the project. The use of project-based team-oriented labwork is recognized by many as providing a simulation of the "real world".[17−21] Although there occasionally is the predictable student complaint of less than equal sharing of the workload, end-of-term peer evaluations, which have a small impact on the student course grades, have all but eliminated this issue.

Finally, with regard to the "cookbook" challenge, while the student handout provides specific details required to conduct the experiment, as the term progresses students begin to encounter instances where they are required to make decisions about how best to proceed with a protocol. For example, the decision about which fractions to combine from the size exclusion column is not straightforward; students need to determine which fractions are most appropriate to combine to maximize recovery without sacrificing purity of their LDH. In addition, performing quantitative assays of final pools requires teams to do preliminary testing to determine the dilution factor and aliquot size to use to optimize the assay. Spectroscopic data must be carefully reviewed to ensure that it is being appropriately evaluated, something that students struggle with early in the term but master by the time they are ready to branch out to do more independent or small group projects at mid-quarter. Project laboratory reports often include viable suggestions about how to improve the procedure and/or potential extensions to the project. Examples of suggestions include (1) testing the use of a "batch" adsorption


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**Table 2. Student Achievement of Learning Goals and Objectives Measured by Formal Written Examination and Laboratory Reports**

| Learning Goal Tested[a] | Assessment Instrument | Learning Objective Assessed | Student Success Rate (SD)[b] |
|---|---|---|---|
| Goal 1 | Written exams | Demonstrate an understanding of the properties of biomolecules that are exploited to effect separations using ion exchange, size exclusion, and affinity chromatography | 92% (14) |
| Goal 2 | Written exams | Describe the criteria for selection of a micropipette for a given application | 94% (16) |
| Goal 2 | Written exams | Explain the role of Coomassie blue dye in the Bradford protein assay and demonstrate an understanding of the sources of error inherent in this assay | 79% (21) |
| Goal 2 | Written exams | Describe the role of NADH in measurement of LDH activity and appreciate the typical sources of error inherent in carrying out an enzyme activity assay | 94% (10) |
| Goal 2 | Laboratory reports | Generate high quality spectrophotometric data and utilize spreadsheets to generate appropriate graphical or tabular representations of the data sets | 89% (17) |
| Goal 3 | Written exams | Describe the role of a variety of reagents in the preparation of SDS gels and gel samples and explain why such gels are able to separate proteins based on differences in molecular size | 95% (13) |
| Goal 3 | Laboratory reports | Utilize digital cameras and computer software to prepare digital images of gels, annotate them, and analyze banding patterns to assess purity of LDH in the 4 purification pools and estimate the molar mass of LDH | 93% (7) |
| Goal 4 | Written exams | Critically evaluate a protocol to detect likely sources of error and make suggestions to improve the procedure | 91% (17) |
| Goal 4 | Laboratory reports | Draw conclusions regarding the overall effectiveness of the purification suite, drawing data from a variety of sources such as the purification table and SDS gel banding patterns | 92% (18) |
| Goal 5 | Laboratory reports | Properly interpret a MSDS to determine the hazard class(es) of reagents used in each protocol and use this information in the preparation of an SOP (standard operating procedure) for one of the procedures used this term. | 98% (4) |

[a]Learning Goals are listed in Box 1 in the Introduction. [b]In the last two offerings of the course, weekly quizzes and a written midterm, with questions keyed to the experiment, were utilized to better assess student learning. The data presented represent the average aggregate scores for those two terms of the course (14−16 students per term).

onto CM-Sephadex in lieu of the column chromatographic separation, (2) testing the impact on recovery and fold purification of skipping the gel filtration step of the suite, and (3) using silver staining of SDS gels to increase the sensitivity of staining to visualize contaminants that may be present in very small quantities. These and other suggested variations indicate that students are well on the way to making the transition from cookbook chef to independent scientist, able to adopt and adapt methodology from the scientific literature for use in new projects.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00713.

Student handouts and instructor notes including detailed information on reagent preparation, CAS numbers, color photographs of electrophoresis gels and blots, sample exam questions, sample grading rubrics for exam questions and lab reports, and information regarding hazards (PDF, DOCX)

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### Notes

The authors declare no competing financial interest.

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