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Edvo-Kit # 153

Edvo-Kit #153

Determination of Protein Molecular Weight

Experiment Objective:

The objective of this experiment is to develop an understanding of protein structure and to determine the molecular weight of unknown prestained proteins by denaturing SDS-polyacrylamide gel electrophoresis.

See page 3 for storage instructions.

Version 153.111202

SAMPLE LITERATURE
Please refer to included weblink for correct version

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Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets

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Experiment Components

	Component	Storage	Check (✓)
A	Standard Protein Markers	-20°C Freezer with desiccant	❑
B	Unknown Prestained Protein #1	-20°C Freezer with desiccant	❑
C	Unknown Prestained Protein #2	-20°C Freezer with desiccant	❑
D	Unknown Prestained Protein #3	-20°C Freezer with desiccant	❑

This kit is designed for six (6) groups sharing three polyacrylamide gels.

All remaining components can be stored at room temperature.

- Tris-Glycine-SDS buffer (10x) ❑
- Practice Gel Loading Solution ❑
- FlashBlue™ Protein Stain Powder ❑
- Transfer Pipets ❑

Experiment Requirements *(NOT included with this experiment)*

- Vertical electrophoresis apparatus (EDVOTEK® Cat. #581 highly recommended)
- D.C. power supply
- Precast 12% SDS polyacrylamide gels (3)
- Micropipette and tips (Cat #638 Fine Tip Micropipette Tips recommended)
- Microwave or Hot plate
- Distilled or deionized water
- Beakers
- Aluminum foil or foam waterbath float
- White Vinegar
- Ethanol (95% or higher)
- 750 mL or 1 L flask or beaker
- Thin spatula or flat blade screw driver
- Small plastic tray or large weigh boat
- Plastic wrap
- White light box (recommended)
- Rocking platform (recommended)
- Metric rulers

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

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Background Information

Proteins are a highly diversified class of biomolecules. Differences in their chemical properties, such as charge, functional groups, shape, size and solubility enable them to perform many biological functions. These functions include enzyme catalysis, metabolic regulation, binding and transport of small molecules, gene regulation, immunological defense and cell structure. Determination of the molecular weight of a protein is of fundamental importance to its biochemical characterization. If the amino acid composition or sequence is known, the exact molecular weight of a polypeptide can be calculated. This assumes that the protein does not contain any "non-amino acid" chemical groups (heme, zinc, covalently bonded carbohydrate, etc.) or that the amount of these groups, if present, is already known. SDS gel electrophoresis is commonly used to obtain reliable molecular weight estimates for denatured polypeptides. Other techniques for the determination of very accurate molecular weights include analytical ultracentrifugation and light scattering. However, these methods require large amounts of highly purified proteins and costly, sophisticated equipment.

A protein can have a net negative or net positive charge, depending on its amino acid composition and the pH. At certain pH values of solutions, the molecule can be electrically neutral, i.e. negative and positive charges are balanced. In this case, the protein is isoelectric. In the presence of an electrical field, proteins with net charges will migrate towards the electrodes of opposite charge.

Proteins exhibit different three-dimensional shapes and folding patterns which are determined by their amino acid sequences and intracellular processing. The precise three-dimensional configuration of a protein is critical to its function. The shapes these molecules can have are spherical, elliptical or rod-like. The molecular weight is a function of the number and type of amino acids in the polypeptide chain. Proteins can consist of a single polypeptide or several polypeptides specifically associated with each other. Proteins that are in their normal, biologically active forms are called native.

The physical-chemical properties of proteins affect the way they migrate during gel electrophoresis. Gels used in electrophoresis (e.g. agarose, polyacrylamide) consist of microscopic pores of a defined size range that act as a molecular sieve. Only molecules with net charge will migrate through the gel when it is in an electric field. Small molecules pass through the pores more easily than large ones. Molecules having more charge than others of the same shape and size will migrate faster. Molecules of the same mass and charge can have different shapes. In such cases, those with more compact shape (sphere-like) will migrate through the gel more rapidly than those with an elongated shape, like a rod. In summary, the charge, size and shape of a native protein all affect its electrophoretic migration rates. Electrophoresis of native proteins is useful in the clinical and immunological analysis of complex biological samples, such as serum, but is not reliable to estimate molecular weights.

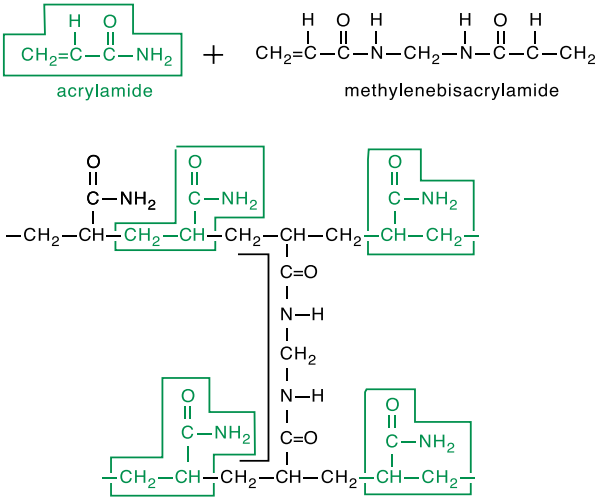

Figure 1:
Schematic of polyacrylamide polymer formation.

POLYACRYLAMIDE GEL ELECTROPHORESIS

Polyacrylamide gels are formed by mixing the monomer, acrylamide, the cross-linking agent, methylenebisacrylamide, and a free radical generator, ammonium persulfate, in aqueous buffer. Free radical polymerization of the acrylamide occurs. At various points the acrylamide polymers are bridged to each other (as shown in Figure 1). The pore size in polyacrylamide gels is controlled by the gel concentration and the degree of

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polymer cross-linking. The electrophoretic mobility of the proteins is affected by the gel concentration. Higher percentage gels are more suitable for the separation of smaller polypeptides. Polyacrylamide gels can also be prepared to have a gradient of gel concentrations. Typically the top of the gel (under the sample wells) has a concentration of 5%, increasing linearly to 20% at the bottom. Gradient gels can be useful in separating protein mixtures that cover a large range of molecular weights. Gels of homogeneous concentration (such as those used in this experiment) are better for achieving wider separations of proteins that occupy narrow ranges of molecular weights.

It should be noted that acrylamide is a neurotoxin and can be absorbed through the skin. However, in the polymerized polyacrylamide form it is non-toxic. The polymerization process is inhibited by oxygen. Consequently, polyacrylamide gels are most often prepared between glass plates separated by strips called spacers. As the liquid acrylamide mixture is poured between the plates, air is displaced and polymerization proceeds more rapidly.

Sodium dodecylsulfate (SDS) is a detergent which consists of a hydrocarbon chain bonded to a highly negatively charged sulfate group (as shown in Figure 2).

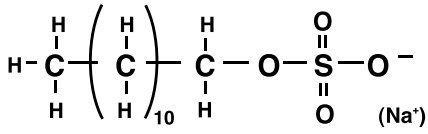

Figure 2:
The chemical structure of sodium dodecylsulfate (SDS).

SDS binds strongly to most proteins and causes them to unfold to a random, rod-like chains. No covalent bonds are broken in this process. Therefore, the amino acid composition and sequence remains the same. Since its specific three-dimensional shape is abolished, the protein no longer possesses biological activity. Proteins that have lost their specific folding patterns and biological activity but have their intact polypeptide chains are called denatured. Proteins which contain several polypeptide chains that are associated only by noncovalent forces will be dissociated by SDS into separate, denatured polypeptide chains. Proteins can contain covalent crosslinks known as disulfide bonds. These bonds are formed between two cysteine amino acid residues that can be located in the same or different polypeptide chains. High concentrations of reducing agents, such as bmercaptoethanol, can break disulfide bonds. This allows SDS to completely dissociate and denature the protein. Proteins that retain their disulfide links bind less SDS, causing anomalous migration. Figure 3 illustrates a protein containing two differently sized polypeptide chains that are cross-linked by a disulfide bond. The chains are also associated by noncovalent forces. The circles represent the native structure.

Certain membrane proteins form SDS complexes that do not contain the usual ratio of detergent, causing anomalous migration rates. Proteins that are highly glycosylated also exhibit anomalous behavior, particularly if the carbohydrate units contain charged groups. It should be noted that SDS does not interact with polysaccharides and nucleic acids.

In most cases, SDS binds to proteins in a constant ratio of 1.4 grams of SDS per gram of protein. On average, the number of bound SDS molecules is half the number of amino acid residues in the polypeptide. The amount of negative charge of the SDS is much more than the negative and positive charges of the amino acid residues. The large quantity of bound SDS efficiently masks the intrinsic changes in the protein. Consequently, SDS denatured proteins are net negative and since the binding of the detergent is proportional to the mass of the protein, the charge to mass ratio is constant. The shape of SDS

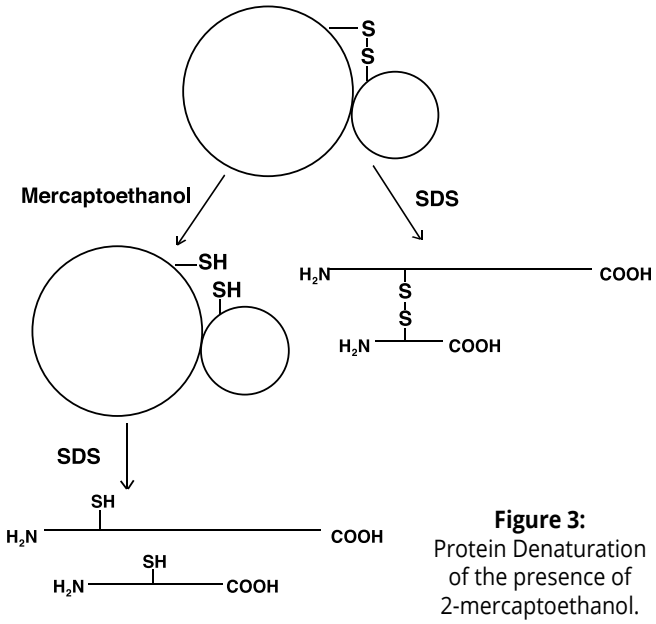

Figure 3:
Protein Denaturation of the presence of 2-mercaptoethanol.

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denatured proteins are all rod-like. The linear size of the rod-like chains is the physical difference between SDS denatured proteins. The larger the molecular weight of the polypeptide the longer the rod-like chain. The electrophoresis gel pores distinguish these size differences.

During SDS electrophoresis, the proteins migrate through the gel towards the positive electrode at a rate that is inversely proportional to their molecular weight. In other words, the smaller the denatured polypeptide, the faster it migrates. The molecular weight of an unknown polypeptide is obtained by the comparison of its position after electrophoresis to the positions of standard SDS denatured proteins. The molecular weights of the standard proteins have been previously determined. After proteins are visualized by staining and destaining, their migration distance is measured. The $\log_{10}$ of the molecular weights of the standard proteins are plotted versus their migration distance. Taking the logarithm Rf allows the data to be plotted as a straight line. The molecular weight of unknowns are then easily calculated from the standard curve.

PROTEIN SAMPLES FOR THIS EXPERIMENT

Standard Protein Markers (Lanes 1 and 6) are a mixture of proteins that give the following denatured molecular weights: prestained 94,000; 67,000; 38,000; 30,000; 20,000; and 14,000 Da. The denatured values have been rounded off for convenience in graphical analysis.

The protein samples have been denatured with the anionic detergent sodium dodecyl sulfate (SDS). Under the experimental conditions, the proteins will have a mobility in the gel that is inversely proportional to the logarithm of their molecular weights. Proteins of known molecular weights will be separated by electrophoresis in parallel and will be used to estimate the molecular weights of the unknown protein samples by graphical analysis. All protein samples contain buffer, SDS, b-mercaptoethanol as the reducing agent for disulfide bonds, glycerol to create density greater than that of the electrode buffer and the negatively charged tracking dye bromophenol blue. The tracking dye will migrate ahead of the smallest proteins in these samples towards the positive bottom electrode. The molecular weight estimates obtained from SDS polyacrylamide gel electrophoresis are of denatured proteins. Since proteins often consist of multiple subunits (polypeptide chains), subunit molecular weight information will be obtained. The molecular weights of the proteins in their native states will be provided so that the number of subunits can be determined. The protein samples provided have been purified to approximately 80-90% purity by salt fractionation and column chromatography procedures. Minor protein bands may appear which may be due to aggregation or contamination.

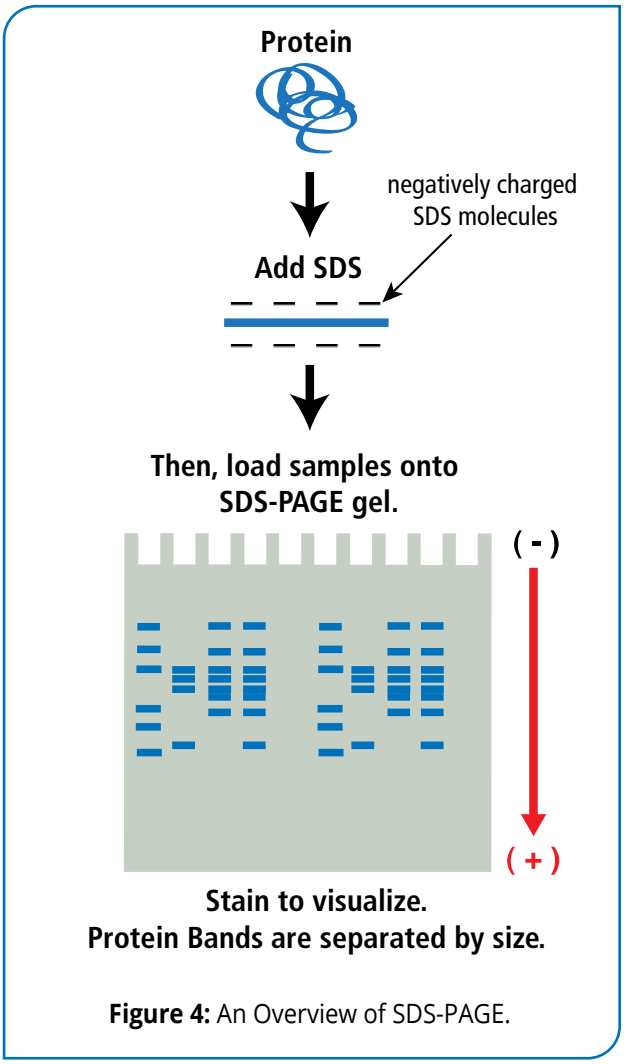

Figure 4: An Overview of SDS-PAGE.

Since the proteins are prestained, the individual bands will be visible during electrophoresis. After electrophoresis, preliminary measurements can be made without removing the gel from the plastic cassette. The prestained proteins can be made more visible by placing the gel in staining solution (Figure 4). The proteins are usually precipitated and in the gel matrix during the staining procedure by a process called fixation. Fixation is necessary to prevent protein diffusion, which causes blurry bands and reduced intensity.

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Experiment Overview

EXPERIMENT OBJECTIVE:

The objective of this experiment is to develop an understanding of protein structure and to determine the molecular weight of unknown prestained proteins by denaturing SDS-polyacrylamide gel electrophoresis.

LABORATORY SAFETY:

Be sure to READ and UNDERSTAND the instructions completely BEFORE starting the experiment. If you are unsure of something, ASK YOUR INSTRUCTOR!

- Wear gloves and goggles while working in the laboratory.
- Exercise caution when working in the laboratory – you will be using equipment that can be dangerous if used incorrectly.
- Wear protective gloves when working with hot reagents like boiling water and melted agarose.
- DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
- Always wash hands thoroughly with soap and water after working in the laboratory.

Wear gloves and safety goggles

Acrylamide is a known neurotoxin and carcinogen and should be handled with extreme caution. Liquid acrylamide, used in the manufacture of SDS-PAGE gels, should only be handled in a chemical fume hood while wearing gloves and goggles. Polymerized acrylamide, including precast acrylamide gels, is safe but should still be handled with caution at all times.

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

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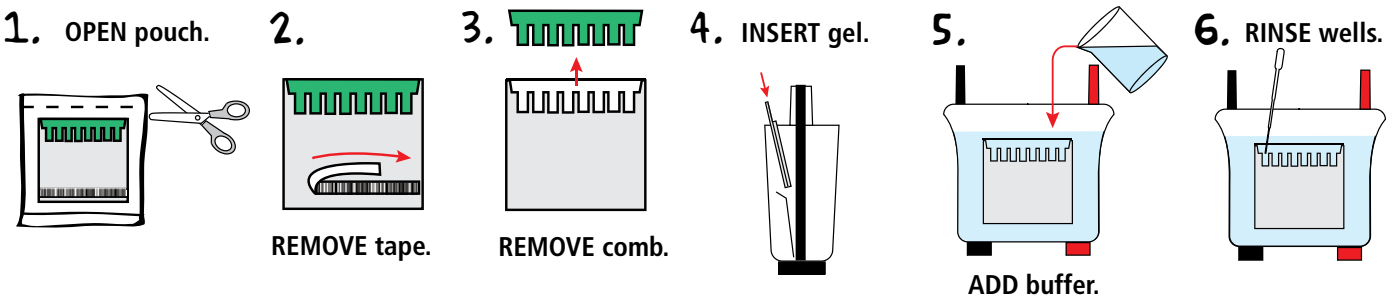
Module I-A: Preparing Precast Polyacrylamide Gels For Electrophoresis

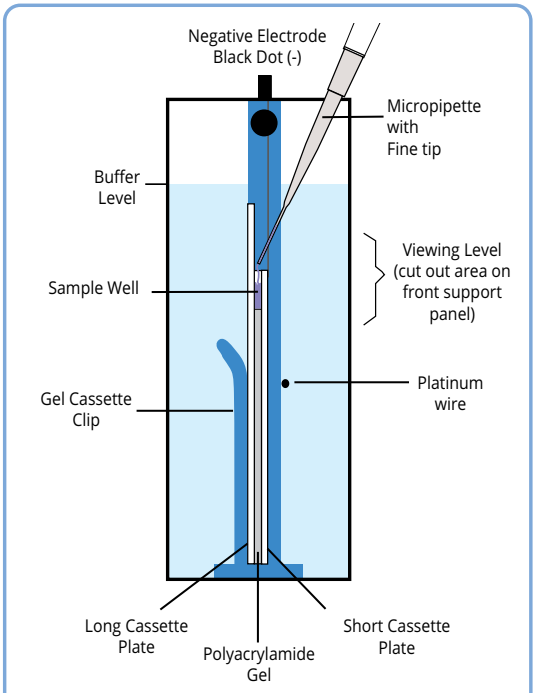


PREPARING THE POLYACRYLAMIDE GEL AND CHAMBER

NOTE: Although precast polyacrylamide gels and protein chambers will vary slightly in design, the procedure for their use will be similar.

1. **OPEN** the pouch containing the gel cassette. Remove the cassette and place on bench with the shorter front plate facing up.
2. Gels may feature a sticker or tape at the bottom of the front plate. **REMOVE** the tape (if present) to expose the bottom of the gel.
3. Carefully **REMOVE** the comb by gently pulling upwards. Pull the comb straight up to prevent damage to the wells of the gel.
4. **INSERT** the gel into the electrophoresis chamber. Orient the gel according to the manufacturer's instructions. ***NOTE: For EDVOTEK® vertical electrophoresis chambers, the short plate should face the middle of the apparatus.***
5. **ADD** diluted electrophoresis buffer to the chamber. The buffer should cover the top of the shorter plate.
6. **RINSE** each well by squirting electrophoresis buffer into the wells using a transfer pipet. Using the transfer pipet, carefully straighten any wells which may have been distorted during comb removal or rinsing.

The gel is now ready for practice gel loading.



A polyacrylamide gel cassette in the EDVOTEK® Vertical Electrophoresis Apparatus, Model #MV10.

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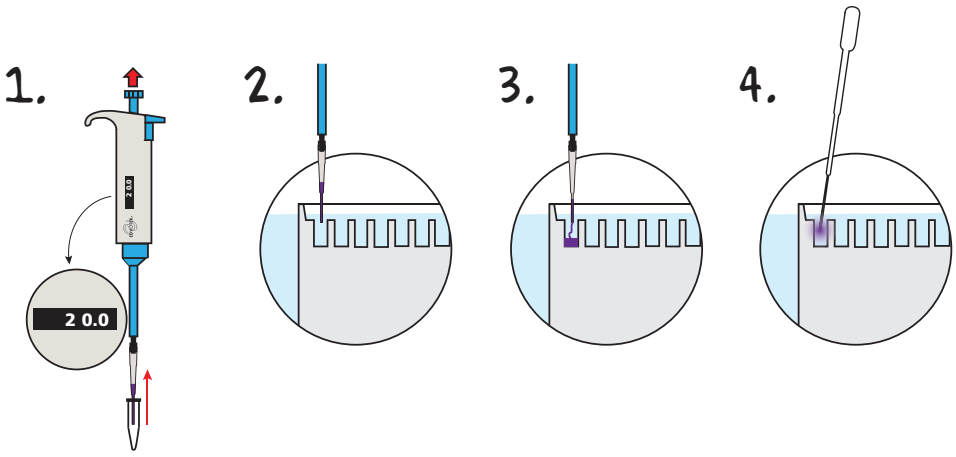
Module I-B: Practice Gel Loading (OPTIONAL)



Wear gloves and safety goggles

NOTE: EDVOTEK® Cat. #638, Fine Tip Micropipette Tips are recommended for loading samples into polyacrylamide gels. A regular microtip may damage the cassette and result in the loss of protein samples.

1. **PLACE** a fresh tip on the micropipette. **REMOVE** 20 µL of practice gel loading solution.

2. **PLACE** the lower portion of the pipette tip below the surface of the electrode buffer, directly over a sample well. The tip should be at an angle pointed towards the well. The tip should be partially against the back plate of the gel cassette, but the tip opening should be over the sample well. **Do not try to jam the pipette tip in between the plates of the gel cassette.**

3. **EJECT** all the sample by steadily pressing down on the plunger of the automatic pipette. Do not release the plunger before all the sample is ejected. Premature release of the plunger will cause buffer to mix with sample in the micropipette tip. Release the pipette plunger after the sample has been delivered and the pipette tip is out of the buffer.

4. **REMOVE** the practice gel loading solution from the sample wells. **FILL** a transfer pipet with buffer and **SQUIRT** a stream into the sample wells. This will displace the practice gel loading solution, which will be diluted into the buffer and will not interfere with the experiment. ***NOTE: Practice gel loading solution must be removed from the sample wells prior to sample loading.***

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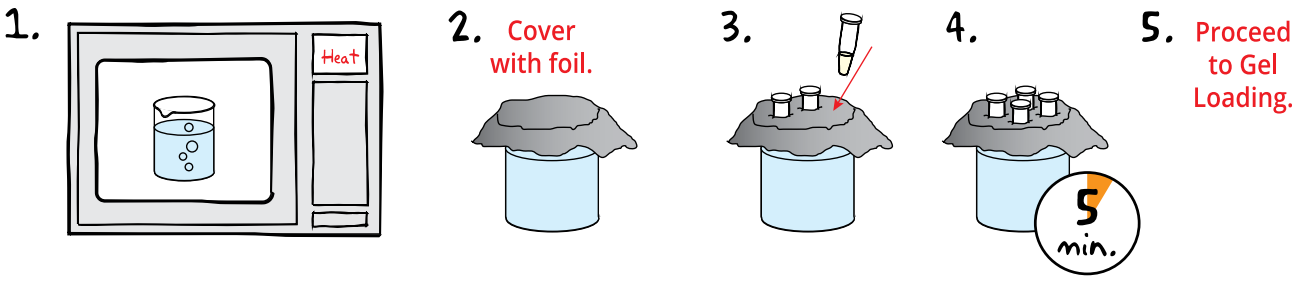
Module II: Performing SDS-PAGE with Protein Samples



PROTEIN DENATURATION:

NOTE: PROCEED to gel loading if your lab instructor has already heated the protein samples.

1. Using a hot plate or microwave, **HEAT** a beaker of water until it boils.
2. **COVER** with aluminum foil and carefully remove from heat.
3. Tightly **CAP** sample tubes. **PUSH** tubes A-D through foil to suspend in the boiling water.
4. **INCUBATE** the samples for 5 minutes.
5. Immediately **PROCEED** to loading the gel while the samples are still warm.

Wear gloves and safety goggles

FREEZING PROTEINS: Unused portions of the protein samples can be frozen for later use. When needed, repeat steps 1-4 and proceed to gel loading.

Samples must be boiled in screw top microcentrifuge tubes!

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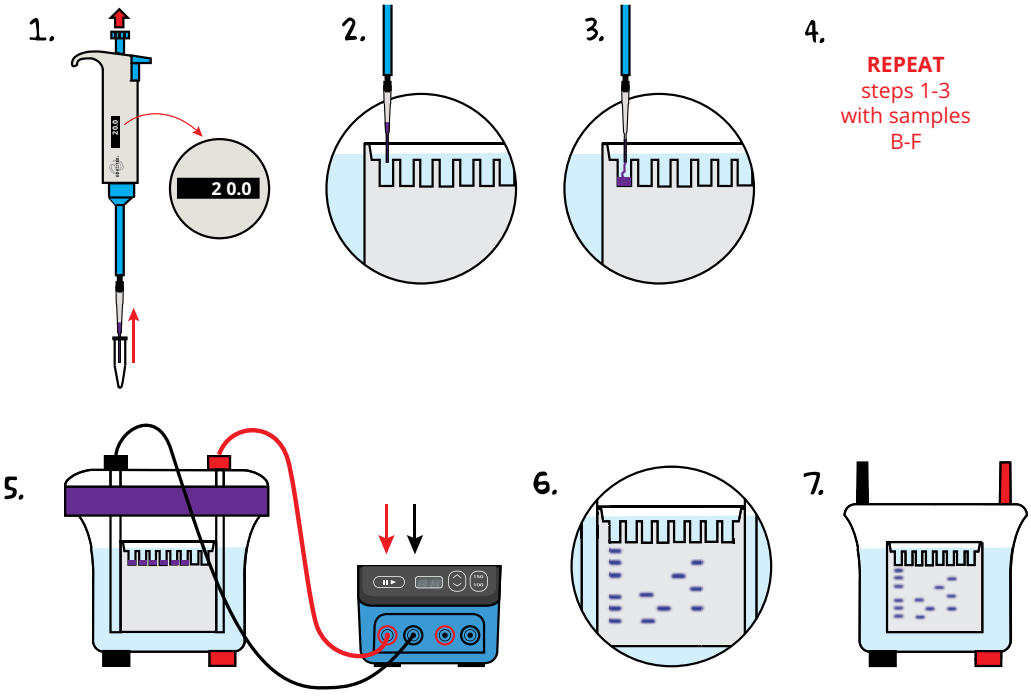
Module II: Performing SDS-PAGE on Protein Samples, continued



LOADING THE PROTEIN SAMPLES:

1. Using a fresh pipette tip, **MEASURE** 20 µL of the Standard Protein Marker (A).
2. **PLACE** the pipette tip under the buffer and directly above the sample well, resting gently against the back plate of the gel cassette.
3. Slowly **DISPENSE** the sample by depressing the plunger.
4. **REPEAT** steps 1-3 with protein samples B-D, changing the tip between each new sample.
5. Once all samples have been loaded, carefully **PLACE** the cover onto the electrode terminals and **CONNECT** the electrical leads to the power supply.
6. **SET** the voltage of the power supply and **PERFORM** electrophoresis (See Table A for time and voltage guidelines). Allow the proteins to separate on the gel for the recommended length of time, or until the tracking dye reaches the bottom of the gel.
7. **TURN OFF** the power supply and carefully **REMOVE** the lid. The gel can now be removed from the chamber and stained.

TABLE 1: GEL LOADING

Lane 1	Tube A	Standard Protein Markers (Group 1)
2	Tube B	Unknown protein #1 (Group 1)
3	Tube C	Unknown protein #2 (Group 1)
4	Tube D	Unknown protein #3 (Group 1)
5	---	---
6	Tube A	Standard Protein Marker (Group 2)
7	Tube B	Unknown protein #1 (Group 2)
8	Tube C	Unknown protein #2 (Group 2)
9	Tube D	Unknown protein #3 (Group 2)
10	---	---

Table A — Time and Voltage Guidelines

	Recommended Time	
Volts	Minimum	Optimal
100	80 min.	95 min.
125	60 min.	75 min.
150	50 min.	60 min.

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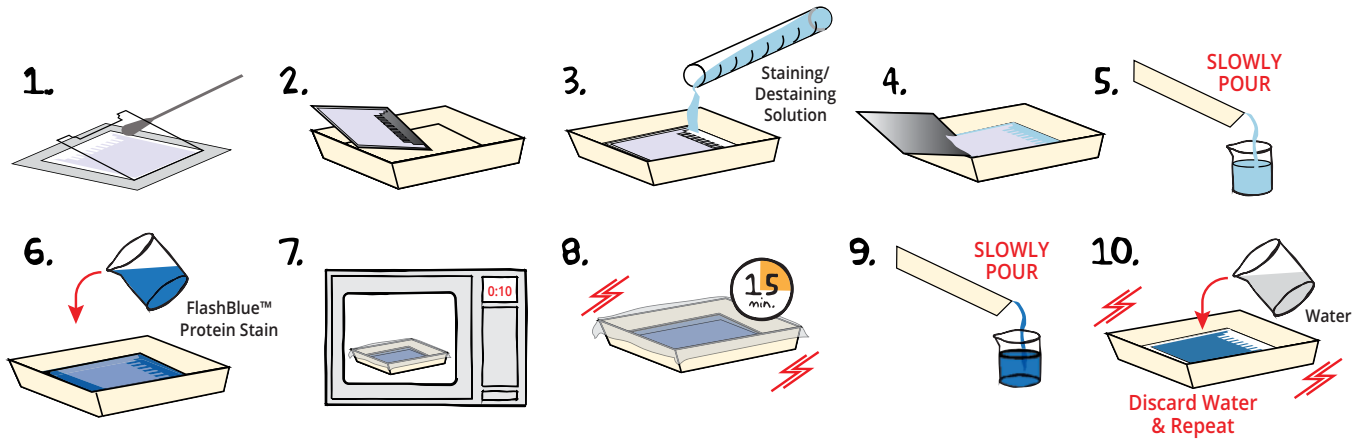
Module III: Staining with FlashBlue™ Protein Stain (OPTIONAL)

Although the protein samples are provided in a pre-stained format it is possible to increase the intensity of the bands by using FlashBlue™ Protein Stain. Staining is rapid and sensitive. Student groups that shared a polyacrylamide gel during electrophoresis should also stain this gel together.



1. After electrophoresis, **LAY** the cassette down and **REMOVE** the front plate by placing a thin spatula or screwdriver at the side edge and gently lift it away from the larger back plate. In most cases, the gel will stay on the back plate. If it partially pulls away with the front plate, let it fall onto the back plate. ***Handle very carefully as the thin gels are extremely fragile.***

2. **TRANSFER** the gel on the back plate to a clean tray.

3. **ADD** a sufficient volume (approximately 50-75 mL) of the staining/destaining solution into the tray to **COVER** the gel and back plate.

4. Carefully **REMOVE** the back plate from the tray, leaving just the gel in the tray containing the staining/destaining solution. Bands may be easier to see once the cassette is removed. **OBSERVE** the gel and take a photo/sketch the banding pattern in your notebook before continuing. ***NOTE: If the gel sticks to the plate, gently nudge the gel off the plate using two GLOVED fingers.***

WEAR GLOVES AND SAFETY GOGGLES

Gloves must be worn during this procedure. Avoid touching the gel without gloves.

Polyacrylamide gels are very thin and fragile. Use care in handling to avoid tearing the gel.

5. **DISCARD** the staining/destaining solution. ***Pour slowly to keep the gel in the container.***

6. **ADD** 30 mL of prepared FlashBlue™ Protein Stain.

7. (OPTIONAL) **COVER** the container with plastic wrap and **MICROWAVE** for 10 seconds to gently heat the solution.

8. **INCUBATE** for 15 minutes at room temperature, **SHAKING** occasionally.

9. **DISCARD** the FlashBlue™ Protein Stain solution. ***Pour slowly to keep the gel in the container.***

10. **WASH** the gel by partially filling container with water and gently rocking back and forth several times. **DISCARD** the used water and **REPEAT** with fresh water.

continued

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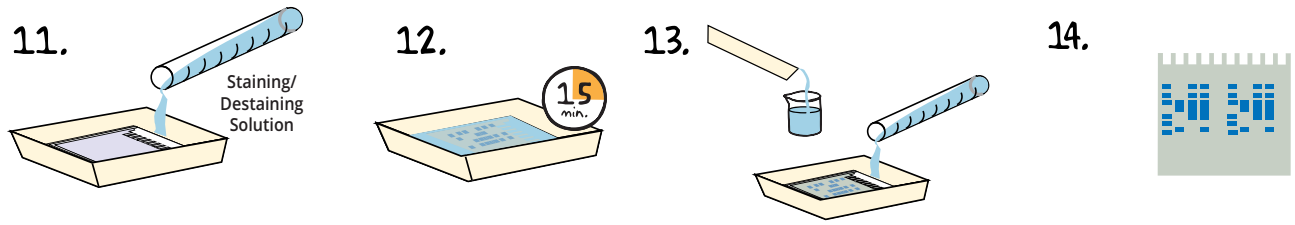
Module III: Staining with FlashBlue™ Protein Stain (OPTIONAL), continued



11. **ADD** 30 mL of staining/destaining solution to the gel.

12. **INCUBATE** for 15 minutes at room temperature. **EXAMINE** the gel.

13. (OPTIONAL) **DISCARD** the used staining/destaining solution and **ADD** an additional 30 mL of staining/destaining solution. **INCUBATE** for 15-60 minutes at room temperature until the appearance and contrast of the protein bands against the background improves.

14. After staining, protein bands will appear medium to dark blue against a light background. A white light box can be used to better visualize the protein bands. **PHOTOGRAPH** results.

STORING THE GEL

- Gel may be left in deionized water for several hours with no loss in sensitivity and band intensity. This step should be performed once a desired background and stained protein bands are obtained. Pour off the destaining solution from Step 12 (or 13) and add a sufficient amount of deionized water to cover the gel.

- For permanent storage, the gel can be dried between two sheets of cellophane (saran wrap) stretched in an embroidery hoop. Air dry the gel for several days until the gel is paper thin. Cut the "extra" saran wrap surrounding the dried gel. Place the dried gel overnight between two heavy books to avoid curling. Tape it into a laboratory book.

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Module IV: Determination of Molecular Weights

	Migration Distance (cm)	Molecular Weight (daltons)
Marker Protein 1 (Phosphorylase)		94,000
Marker Protein 2 (Bovine Serum Albumin)		67,000
Marker Protein 3 (Ovalbumin)		38,000
Marker Protein 4 (Carbonic anhydrase)		30,000
Marker Protein 5 (Soybean Trypsin Inhibitor)		20,000
Marker Protein 6 (Lysozyme)		14,000
Unknown Protein Sample 1		
Unknown Protein Sample 2		
Unknown Protein Sample 3		

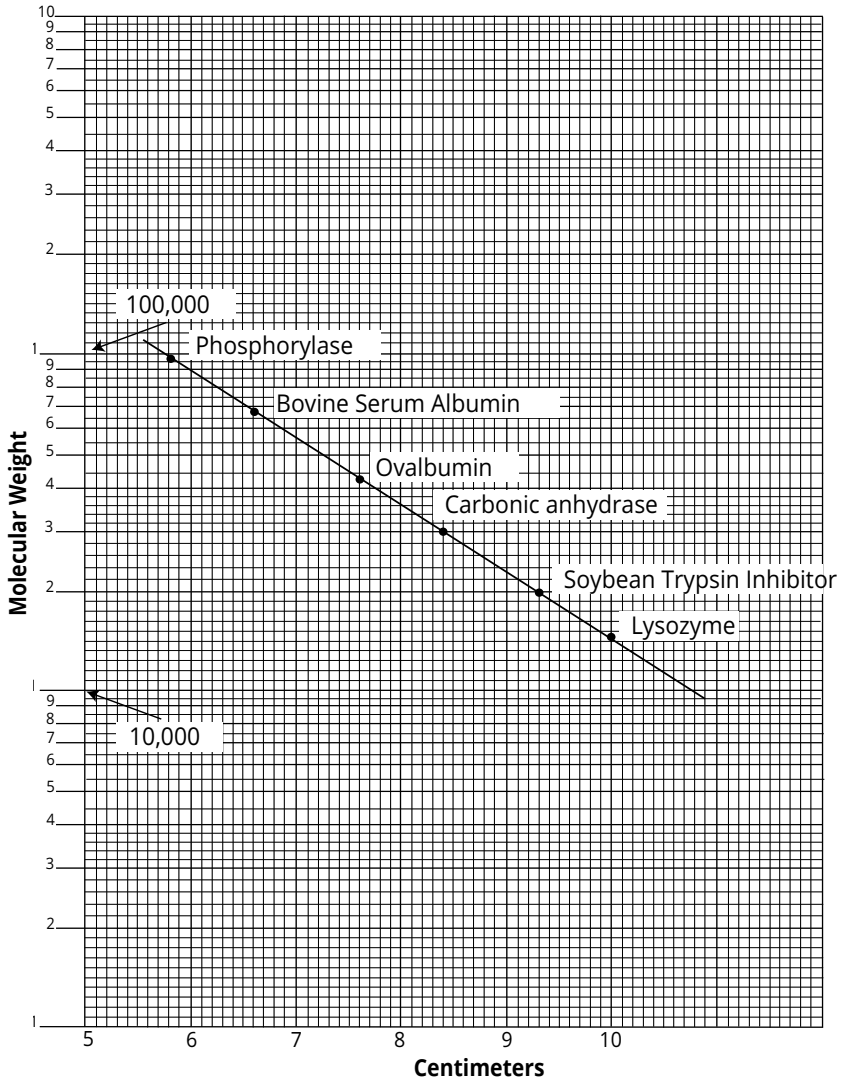
1. **MEASURE** the migration distance, in centimeters (to the nearest millimeter) of every major band in the gel. All measurements should be from the bottom of the sample well to the bottom of the protein band.

2. Using semilog graph paper, **PLOT** the migration distance or relative mobility (Rf) of each standard protein on the non-logarithmic x-axis versus its molecular weight on the logarithmic y-axis. **CHOOSE** your scales so that the data points are well spread out.

3. **DRAW** the best average straight line through all the points. This line should roughly have an equal number of points scattered on each side of the line. As an example, refer to the figure at left. This method is a linear approximation.

4. Using your standard graph, **DETERMINE** the molecular weight of the three unknown proteins. This can be done by finding the Rf (or migration distance) of the unknown band on the x-axis and drawing a straight vertical until the standard line is intersected.

5. A straight line is then made from the intersection across to the y-axis where the approximate molecular weight can be determined.



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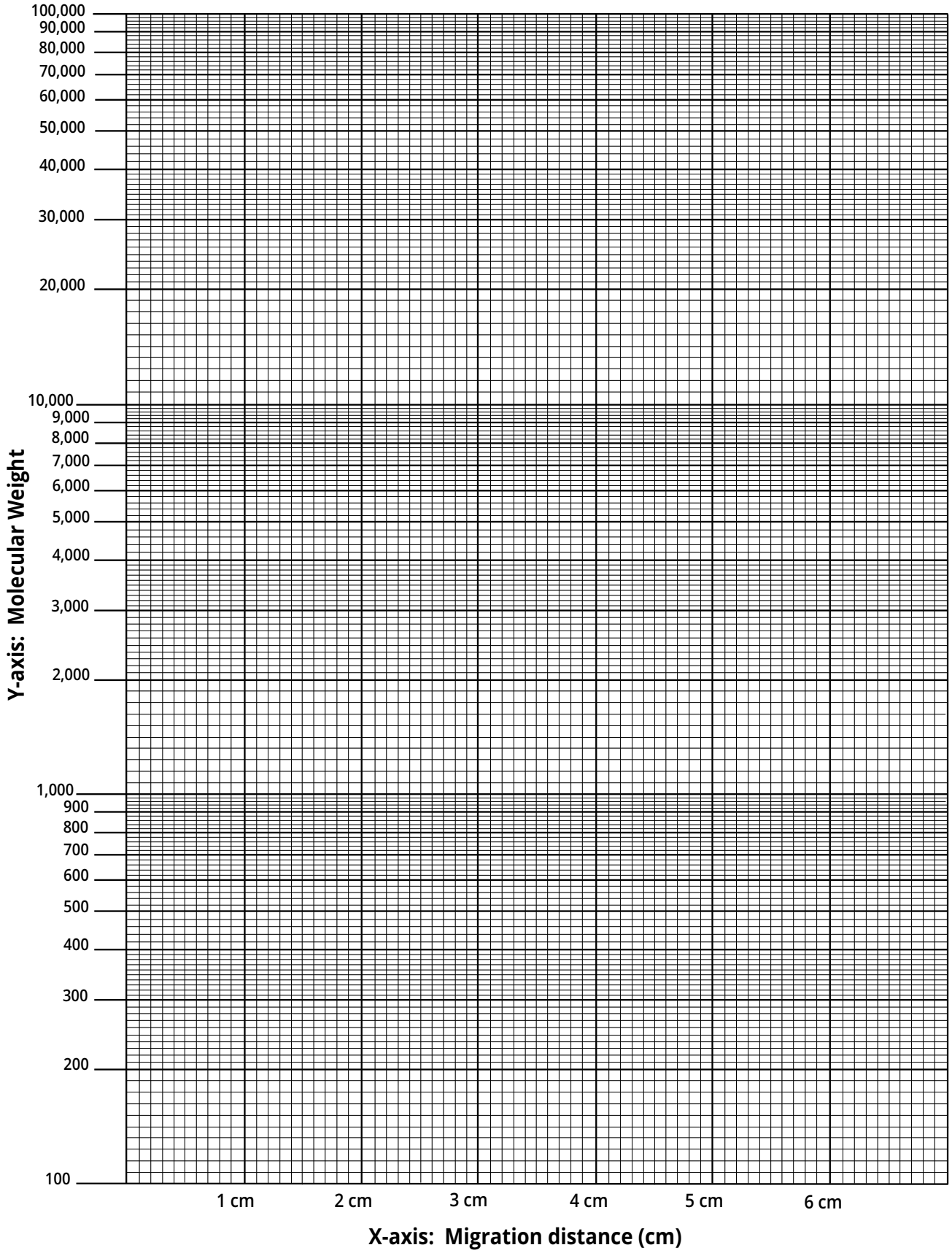

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Study Questions

1. The migration rate of glutamate dehydrogenase is very similar to the migration rate of b-amylase during SDS polyacrylamide gel electrophoresis. Yet the native molecular weight of glutamate dehydrogenase is 330 kDa and that of β-amylase is 206 kDa. Explain.

2. Many genes have been cloned and sequenced. The precise amino acid sequence of a polypeptide can be determined from the DNA sequence and the molecular weight can be calculated. Can a reasonable estimate of the native molecular weight of a protein be determined from the sequence of their structural genes? Why?

3. IgG contains 2 small and 2 large polypeptide chains. A preparation of IgG was incubated with SDS, heated and submitted to SDS polyacrylamide gel electrophoresis. One major band near the top of the gel was observed after staining. Explain.

4. Glutamate dehydrogenase can have a native molecular weight of 2 x 106 in concentrated solutions. Upon the addition of NADH and glutamate, the native molecular weight is 330,000. Explain these phenomena.

5. A purified, active preparation of carbonic anhydrase was submitted to native polyacrylamide gel electrophoresis at alkaline pH. Three major bands were observed after staining. The same preparation of protein was denatured and submitted to SDS-polyacrylamide gel electrophoresis. One band was observed after staining. Explain these results.

6. A glycoprotein consisting of a single polypeptide chain and over 40% (by weight) of N-acetylglucosamine, mannose and sialic acid was found to have a native molecular weight of 75,000 by several analytical methods. However, analysis by SDS polyacrylamide gel electrophoresis gave molecular weight of 100,000. Explain.

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Instructor's Guide

ORGANIZING AND IMPLEMENTING THE EXPERIMENT

Prior to starting this experiment, carefully check the list of components and requirements on page 3 to ensure you have all the necessary components and equipment.

This experiment requires three 12% Polyacrylamide Gels to be shared by the 6 student groups. Each group requires 4 sample wells.

Preparation For:	What to do:	When:	Time Required:
Module I: Preparing Precast Polyacrylamide Gels for Electrophoresis	Prepare diluted electrophoresis buffer	Up to one day before performing the experiment.	15 min.
	Rehydrate and aliquot protein samples	Up to one day before performing the experiment and stored at -20˚ C.	15 min.
Module II: Performing SDS-PAGE on Protein Samples	Prepare waterbaths for denaturing proteins	Up to one day before performing the experiment.	15 min.
	Denature proteins (optional)	No more than 10 min. before performing the experiment.	10 min.
Module III: Gel Staining with FlashBlue™ Protein Stain	Prepare staining solutions	Anytime before performing the experiment.	10 min.

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Pre-Lab Preparations

PREPARING PROTEIN SAMPLES

1. Add 130 µL of distilled or deionized water to each tube (A-D) and allow the samples to hydrate for several minutes. Vortex or flick tube vigorously to mix. Resuspended proteins may be kept at room temperature for immediate use or frozen until needed.
2. The protein samples must be heated in their original 1.5 ml screw-top microcentrifuge tubes before use. This step can be completed by laboratory instructors immediately before the lab period or it can be performed by the students during the lab period. For instructions on denaturing the protein samples please refer to Module II, page 10.
3. Samples can be aliquoted for each of the 6 student groups, or students can share the rehydrated sample stock tubes. Have students load samples onto the polyacrylamide gel while the samples are still warm to avoid aggregation. The volume of sample to load per well is 20 µL.
4. Store any unused portion of reconstituted sample at -20°C and repeat steps 2 and 3 when using samples at a later time.

PREPARING ELECTROPHORESIS BUFFER

Prepare the electrophoresis buffer by adding and mixing 1 part Tris-Glycine-SDS 10x buffer concentrate to 9 parts distilled water.

The approximate volume of 1x electrophoresis buffer required for EDVOTEK Protein Vertical Electrophoresis units are listed in Table B. The buffer should just cover the back plate of the gel cassette.

Table B — Tris-Glycine-SDS Electrophoresis (Chamber) Buffer

EDVOTEK Model #	Total Volume Required	Concentrated Buffer (10x)	+ Distilled Water
MV10	580 mL	58 mL	522 mL
MV20	950 mL	95 mL	855 mL

ELECTROPHORESIS TIME AND VOLTAGE

Your time requirements will dictate the voltage and the length of time it will take for your samples to separate by electrophoresis. Approximate recommended times are listed in Table A.

Run the gel until the bromophenol blue tracking dye is near the bottom edge of the gel.

Table A — Time and Voltage Guidelines

	Recommended Time	
Volts	Minimum	Optimal
100	80 min.	95 min.
125	60 min.	75 min.
150	50 min.	60 min.

PREPARATION FOR STAINING GELS

1. Prepare a stock solution of white vinegar and ethanol* by combining 400 mL white vinegar with 200 mL ethanol. Gently mix. Label as "Staining/Destaining Solution".
2. Add 125 mL of the Staining/Destaining Solution to a 250 mL flask or beaker. Add the entire contents of the FlashBlue™ Protein Stain powder and briefly stir or shake to mix. Residual powder can be rinsed from the tube using an additional 1 mL of Staining/Destaining Solution.
3. Store both solutions at room temperature until needed.
4. TWO student groups will share: 30 mL FlashBlue™ Protein Stain, 140 mL Staining/Destaining Solution, water, a staining tray, and plastic wrap.

**White vinegar, sometimes called distilled or spirit vinegar, is an easy to find cooking and cleaning vinegar with an acetic acid concentration between 5-8% and a pH ~2.6. Ethanol is a common lab supply which is available at various concentrations. Our FlashBlue™ Protein Stain has been designed to work with a wide range of white vinegars. However, we do recommend using 95% Ethanol or higher.*

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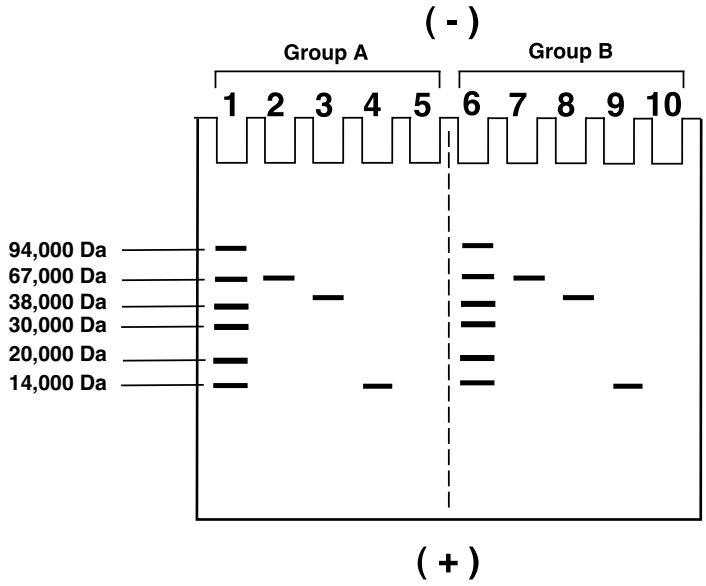
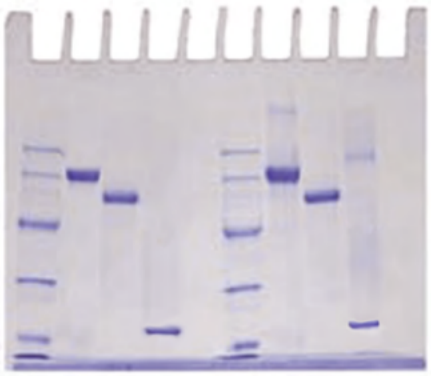
Experiment Results and Analysis

The idealized schematic below shows the relative positions of the denoted protein bands, but are not drawn to scale. Faintly stained bands obtained from some samples are minor contaminants. Do not measure these bands during analysis. Error can be ± 10% by this method.

Lanes	Sample	Protein	Denatured Molecular Weight*
1 & 6	A	Standard ProteinMarkers	See Figure
2 & 7	B	Unknown Protein 1	68,000
3 & 8	C	Unknown Protein 2	58,000
4 & 9	D	Unknown Protein 3	14,000





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Please refer to the kit insert for the Answers to Study Questions

Appendix A

EDVOTEK® Troubleshooting Guide

PROBLEM:	CAUSE:	ANSWER:
Gel is not running properly.	Running buffer was not properly prepared.	Check buffer protocol, make fresh buffer.
	Wrong buffer used.	Check gel recipe, buffer must be compatible with the gel.
	Buffer volume is too low.	Buffer must fully cover the sample wells throughout the entire experiment.
	Gel is inserted in the wrong orientation.	Check with manufacturer for proper setup of the electrophoresis chamber.
	Malfunctioning electrophoresis chamber or power supply.	Consult with manufacturer of electrophoresis chamber or power supply.
	Tape at bottom of precast gel not removed.	Carefully remove tape before running the gel.
	Buffer volume is too low.	Buffer must fully cover the sample wells throughout the entire experiment.
	Electrodes not connected or polarity reversed.	Check electrode connections at the gel box and power supply.
Poor band resolution or separation.	Diffusion of samples before power was turned on.	Minimize time between loading samples and the start of electrophoresis.
	The gel is old or expired.	Make fresh gels or order new pre-cast gels.
	Wrong concentration of acrylamide gel.	The kit is designed for 12% acrylamide gels, other concentrations will affect results.
Smiling or frowing of bands.	Proteins have been overloaded.	EDVOTEK ® has optimized this kit to avoid overloading. Be sure to load the amount recommended by the protocol.
	Wrong buffer was used.	Check gel recipe, the buffer must be compatible with the gel.
	Incorrect voltage supplied to the gel.	Check the protocol for the recommended voltage (page 13).
No bands on gel/ smallest bands missing from gel.	Proteins ran off gel.	Use the appropriate length of time for the chosen voltage. Be sure to monitor the tracking dye while the gel is running. For best results, the tracking dye should run 8-9 cm and should not be allowed to run off the gel.
Proteins have accumulated in the wells of the gel.	Proteins have aggregated.	Ensure proteins have fully denatured; boil proteins for 5 min. and load while still warm.
Bands are smeary and distorted.	The gel has overheated.	Reduce voltage, check buffer concentration and dilute if necessary.
Bands are faint.	Proteins have diffused or faded.	Repeat staining with increased staining times and/or increased destaining times.
	Too little protein was loaded.	EDVOTEK ® has optimized this kit to avoid underloading. Be sure to load the amount recommended by the protocol.

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