



The Biotechnology Education Company®



153
EDVO-Kit #

Determination of Protein Molecular Weight

Storage:

Some components require freezer storage.
See page 3 for details.

EXPERIMENT OBJECTIVES:

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.

All 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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Upon receipt,
freeze
Components A-D.

There is enough of
each sample for six (6)
groups sharing three
polyacrylamide gels.

All components are
intended for educational
research only. They are
not to be used for diag-
nostic or drug purposes,
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LyphoProtein is a
trademark of
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Experiment Components

- | | | |
|---|------------------------------|------------------------|
| A | Standard Protein Markers | Freezer with desiccant |
| B | Unknown Prestained Protein 1 | Freezer with desiccant |
| C | Unknown Prestained Protein 2 | Freezer with desiccant |
| D | Unknown Prestained Protein 3 | Freezer with desiccant |

Storage

Freezer with desiccant
Freezer with desiccant
Freezer with desiccant
Freezer with desiccant

- Tris-glycine-SDS buffer (10x)
- Protein InstaStain®
- Practice gel loading solution
- Semi-log graph paper template
- Transfer pipets

Room temperature
Room temperature

LyphoProtein™ samples are protein samples which are denatured, lyophilized and ready for electrophoresis after rehydration and heating.

None of the components have been prepared from human sources.

Requirements

- MV10 or MV20 vertical electrophoresis apparatus
- D.C. power supply
- Three 12% precast SDS polyacrylamide gels (Cat. #651 or #652)
- Automatic micropipet and tips (Cat. #638 Fine Tip Micropipet Tips)
- Metric rulers
- 500ml graduated cylinder
- Hot plate or burner
- Methanol (150 ml)
- Glycerol (15 ml)
- Distilled or deionized water
- Glacial acetic acid (80 ml)
- Glass staining tray (optional)
- Aluminum foil or microtest tube holder
- Scissors
- Plastic wrap
- Spatula or gel spacer
- 500 gram weight
- White light box
- Small plastic tray or weigh boat
- Photodocumentation system (optional)

Determination of Protein Molecular Weight

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.

POLYACRYLAMIDE GEL ELECTROPHORESIS

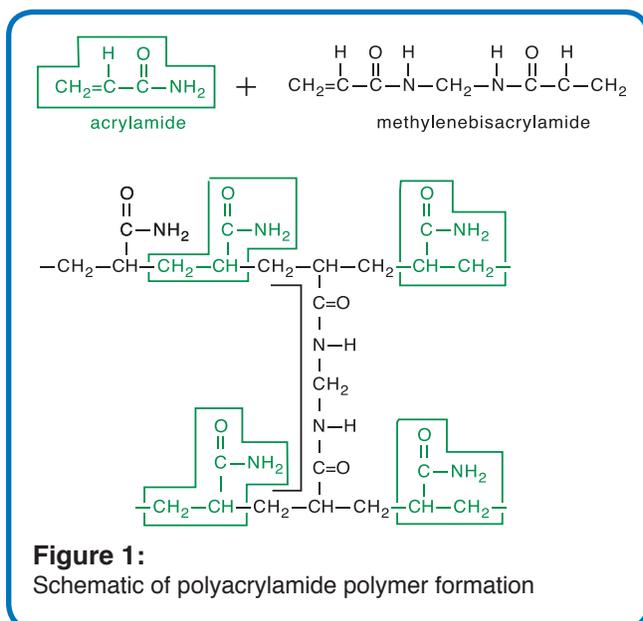
Polyacrylamide gels are formed by mixing the monomer, acrylamide, the cross-linking agent, methylenebisacrylamide, and a free radical generator, ammonium persulfate, in aqueous buffer. Free radical polymerization of the acrylamide occurs. At various points the acrylamide polymers are bridged to each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



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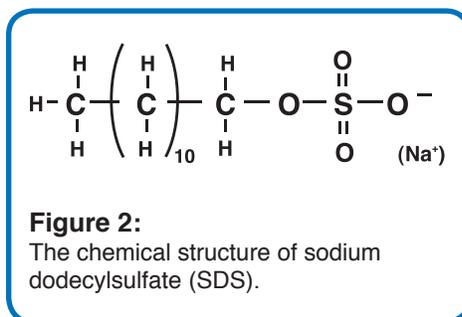
Determination of Protein Molecular Weight

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.

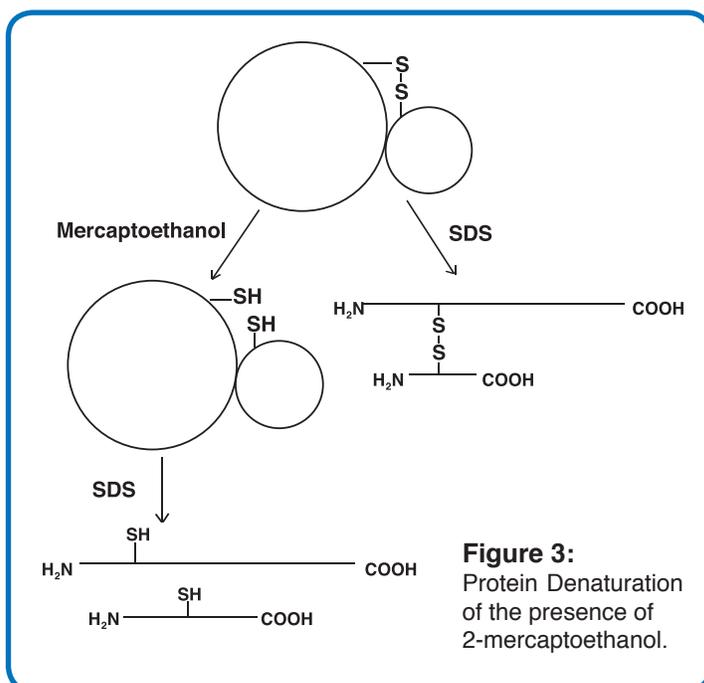
Sodium dodecylsulfate (SDS) is a detergent which consists of a hydrocarbon chain bonded to a highly negatively charged sulfate group (as shown in Figure 2).



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.

Determination of Protein Molecular Weight

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 charges 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 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 R_f allows the data to be plotted as a straight line. The molecular weight of unknowns are then easily calculated from the standard curve.



Determination of Protein Molecular Weight

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.

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. 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. Fixatives often include acetic acid and methanol. Protein InstaStain® is a new state-of-the-art, proprietary patented staining method available exclusively from EDVOTEK®.

Experiment Overview

EXPERIMENT OBJECTIVE:

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

LABORATORY SAFETY

1. Gloves and goggles must be worn at all times.
2. Unpolymerized acrylamide is a neurotoxin and should be handled with extreme caution in a fume hood.
3. Use a pipet pump to measure polyacrylamide gel components. Polymerized acrylamide, such as precast gels, are safe but should still be handled with gloves.



Wear gloves
and safety
goggles



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Protein Denaturation

The samples are denatured proteins which tend to form super-molecular aggregates and insoluble particulates. Heating disrupts metastable aggregates of denatured proteins.

NOTES:

- *If the protein samples (tubes A through D) have not been heated by your lab instructor, follow the heating procedure (Steps 1-2) to heat the samples.*
- *If the protein samples have already been heated by your lab instructor, proceed with *Electrophoresis of Proteins* as outlines on page 10.*

Quick Reference:

The heating (Steps 1-2) disrupts metastable aggregates of denatured proteins. Denatured proteins tend to form super-molecular aggregates and insoluble particulates.

1. Bring a beaker of water, covered with aluminum foil, to a boil. Remove from heat.
2. Make sure the sample tubes A through D are tightly capped and well labeled. The bottom of the tubes should be pushed through the foil and immersed in the boiling water for 5 minutes. The tubes should be kept suspended by the foil.
3. Proceed to loading the gel while the samples are still warm.

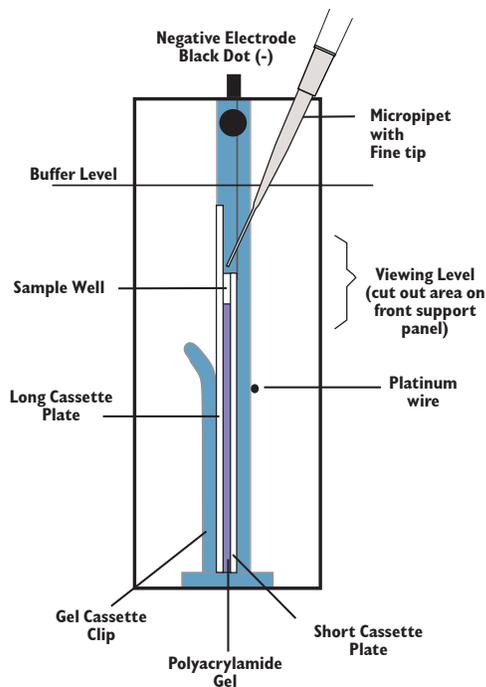
NOTES:

- *Upon completion of loading the samples for electrophoresis, the unused portions of the protein samples can be frozen.*
- *Remove the samples from the freezer and follow steps 1-3, above, to reheat and run the samples when using them at a later time.*

Electrophoresis of Proteins



The figure below shows a polyacrylamide gel cassette in the EDVOTEK® Vertical Electrophoresis Apparatus, Model #MV10.



PREPARING THE POLYACRYLAMIDE GEL FOR ELECTROPHORESIS

Precast Polyacrylamide Gels:

Precast polyacrylamide gels will vary slightly in design. Procedures for their use will be similar.

1. Open the pouch containing the gel cassette with scissors. Remove the cassette and place it on the bench top with the front facing up.

Note: The front plate is smaller (shorter) than the back plate.

2. Some cassettes will have tape at the bottom of the front plate. Remove all of the tape to expose the bottom of the gel to allow electrical contact.
3. Insert the Gel Cassette into the electrophoresis chamber.
4. Remove the comb by placing your thumbs on the ridges and pushing (pressing) upwards, carefully and slowly.

PROPER ORIENTATION OF THE GEL IN THE ELECTROPHORESIS UNIT

1. Place the gel cassette in the electrophoresis unit in the proper orientation. The protein samples will not separate in gels that are not oriented correctly. Follow the directions accompanying the specific apparatus.
2. Add the diluted buffer into the chamber. The sample wells and the back plate of the gel cassette should be submerged under buffer.
3. Rinse each well by squirting electrophoresis buffer into the wells using a transfer pipet.

The gel is now ready for practice gel loading and/or samples.



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Electrophoresis of Proteins

READ ME!

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

PRACTICE GEL LOADING

EDVOTEK® Cat. #638, Fine Tip Micropipet 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 fine tip on the micropipet. Aspirate 20 μ l of practice gel loading solution.
2. Place the lower portion of the fine pipet tip between the two glass plates, 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, as illustrated in the figure on page 10.

Do not try to jam the pipet tip in between the plates of the gel cassette.

4. Eject all the sample by steadily pressing down on the plunger of the automatic pipet.

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 micropipet tip. Release the pipet plunger after the sample has been delivered and the pipet tip is out of the buffer.

5. Before loading protein samples for the actual experiment, the practice gel loading solution must be removed from the sample wells.

Do this by filling a transfer pipet with buffer and squirting 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.

Electrophoresis of Proteins

LOADING PROTEIN SAMPLES

Change pipet tips between loading each sample. Make sure the wells are cleared of all practice loading solution by gently squirting electrophoresis buffer into the wells with a transfer pipet.

Two groups will share each gel. The prestained protein samples should be loaded in the following manner:

Group A

Lane 1	Load 20 μ l of Tube A	Standard Protein Markers
Lane 2	Load 20 μ l of Tube B	unknown protein 1
Lane 3	Load 20 μ l of Tube C	unknown protein 2
Lane 4	Load 20 μ l of Tube D	unknown protein 3

Group B

Lane 6	Load 20 μ l of Tube A	Standard Protein Markers
Lane 7	Load 20 μ l of Tube B	unknown protein 1
Lane 8	Load 20 μ l of Tube C	unknown protein 2
Lane 9	Load 20 μ l of Tube D	unknown protein 3

RUNNING THE GEL

1. After the samples are loaded, carefully snap the cover all the way down onto the electrode terminals. On EDVOTEK electrophoresis units, the black plug in the cover should be on the terminal with the black dot.
2. Insert the plug of the black wire into the black input of the power supply (negative input). Insert the plug of the red wire into the red input of the power supply (positive input).
3. Set the power supply at the required voltage and run the electrophoresis for the length of time as determined by your instructor. When the current is flowing, you should see bubbles forming on the electrodes. The sudsing is due to the SDS in the buffer.
4. After the electrophoresis is finished, turn off power, unplug the unit, disconnect the leads and remove the cover.

Time and Voltage		
Volts	Recommended Time	
	Minimum	Optimal
125	60 min	75 min



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Staining the Gel



Wear gloves
and safety goggles

NOTE:

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

Fixative and Destaining Solution for each gel (100ml)

50 ml	Methanol
10 ml	Glacial Acetic Acid
40 ml	Distilled Water

STORING THE GEL:

Once satisfactory result is achieved, the gel can be stored in distilled or deionized water.

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.

STAINING WITH PROTEIN INSTASTAIN® IN ONE EASY STEP

EDVOTEK features a state-of-the-art, proprietary stain for DNA or Protein gels called InstaStain®. Protein Polyacrylamide gels can be stained with Protein InstaStain® cards in one easy step. Staining is rapid, sensitive and Polyacrylamide gels are ready for visualization in 1-3 hours.

InstaStain® Blue and InstaStain® Ethidium Bromide are also available from EDVOTEK for staining of DNA gels.

1. After electrophoresis, turn off the power and remove the gel cassette from the gel electrophoresis apparatus.
2. To remove the gel from the cassette, lay the cassette down and carefully remove the front plate by placing a coin or a spatula in the slot at the top edge, near the sample wells, and twist to separate the two plates of the cassette.
3. Gently lift the front plate away from the larger back plate. In most cases, the gel will stay on the back plate. If the gel partially sticks to the front plate, let it fall onto the back plate.
4. Pour approximately 100 ml of fixative solution in a small tray.
5. Transfer the back plate of the cassette (with the gel) into the tray containing the fixative solution. Wet gloved fingers with fixative solution and gently nudge the gel off the back plate and remove the plate, leaving the gel submerged in the fixative solution.
6. Gently float a sheet of Protein InstaStain® card with the stain side (blue) facing in the liquid. Remove the Protein InstaStain® card after 30 minutes.
7. Cover the staining tray with saran wrap to prevent evaporation.
8. Gently agitate on a rocking platform for 1-3 hours or overnight.
9. After staining, Protein bands will appear medium to dark blue against a light background* and will be ready for excellent photographic results.

* *Destaining is usually not required but can be carried out if the gel background is too dark. Gels can be destained in several changes of fresh destaining solution until the appearance and contrast of the protein bands against the background improves.*

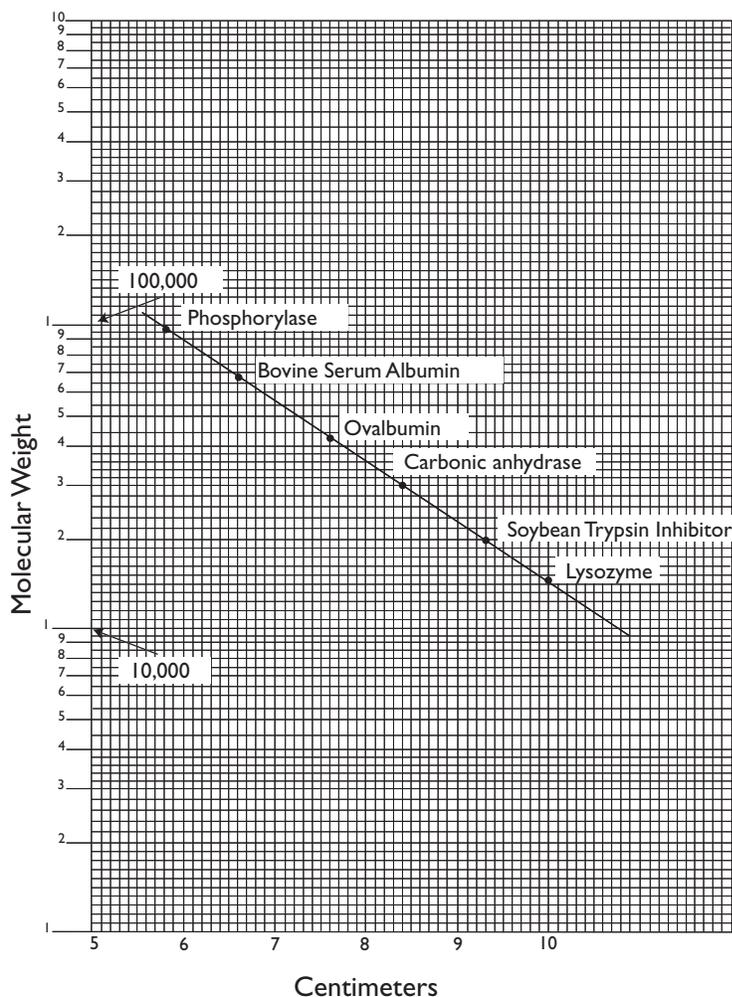
Determination of Molecular Weights

In the example (below), the standard molecular weights are:

94,000	30,000
67,000	20,000
38,000	14,000

If measurements are taken directly from the gel, skip steps 1 and 2.

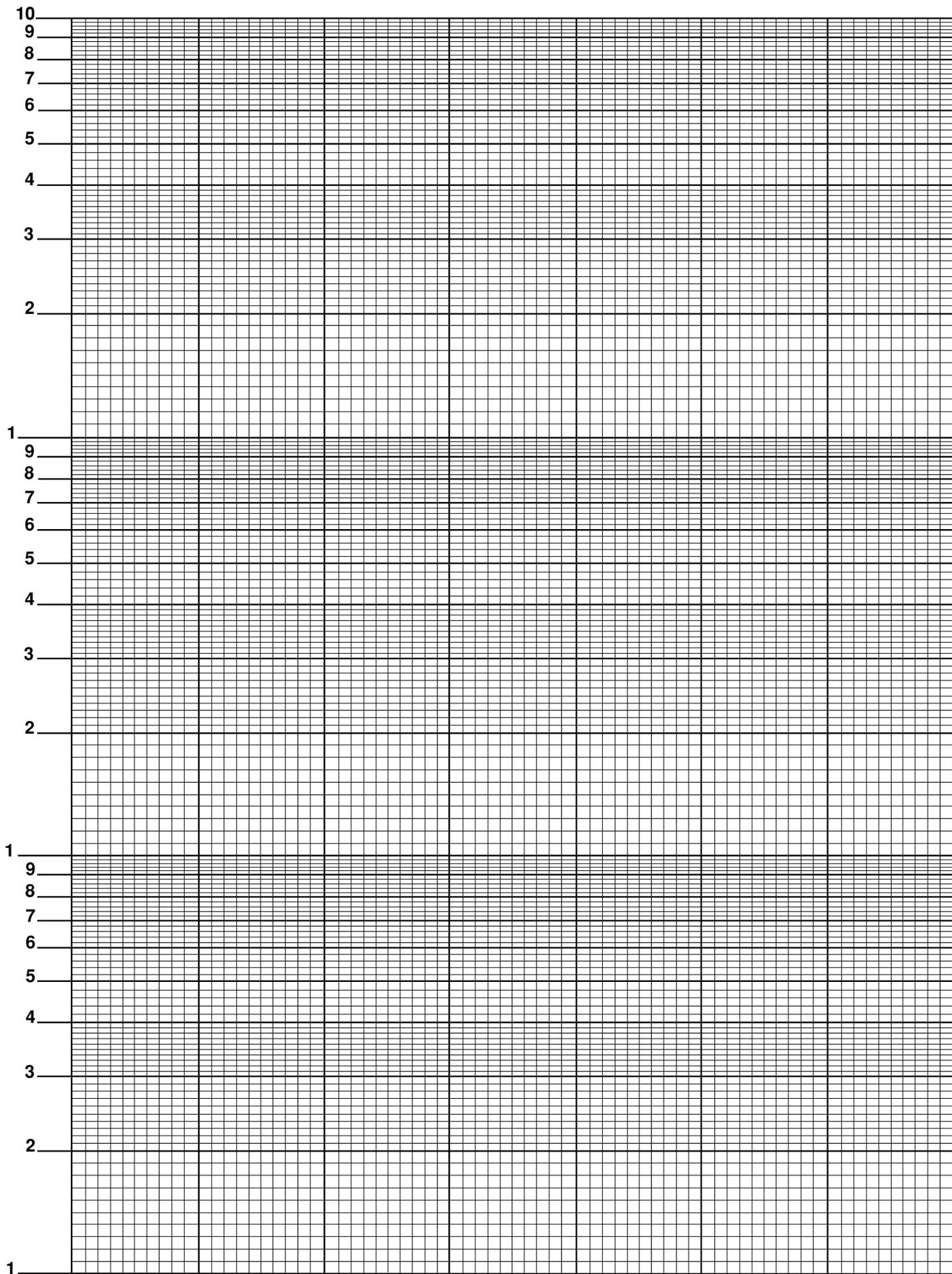
1. Take a transparent sheet, such as cellulose acetate (commonly used with overhead projectors) and lay it over the wrapped gel.
2. With a felt-tip pen, carefully trace the outlines of the sample wells. Then trace over all the protein bands on the gel.
3. Measure the migration distance, in centimeters (to the nearest millimeter) of every major band in the gel. (Ignore the faint bands, refer to Idealized Schematic.) All measurements should be from the bottom of the sample well to the bottom of the protein band.



4. Using semi-log graph paper, plot the migration distance or R_f 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. Assume the second cycle on the y-axis represents 10,000 to 100,000 (see example at left).
5. Draw the best average straight line through all the points. This line should 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.
6. Using your standard graph, determine the molecular weight of the three unknown proteins. This can be done by finding the R_f (or migration distance) of the unknown band on the x-axis and drawing a straight vertical until the standard line is intersected. 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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Study Questions

1. The migration rate of glutamate dehydrogenase is very similar to the migration rate of β -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 2×10^6 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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Notes to the Instructor

If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK® web site. In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

HOW THIS EXPERIMENT IS ORGANIZED

This experiment module contains biologicals and reagents for six (6) groups sharing three (3) polyacrylamide gels (2 groups per gel). Enough buffer is included for three (3) vertical electrophoresis units (Model MV-10 or equivalent). Additional electrophoresis buffer is required for more than three units.

Note: Polyacrylamide gels are not included. You may choose to pour your own polyacrylamide gels or purchase precast gels (Cat. #651 or #652).

The experimental procedures consist of two major parts:

- 1) separation of proteins on polyacrylamide gels,
- 2) determination of molecular weight of unknown proteins.

The staining of protein bands can be conducted using Protein InstaStain®, a new state-of-the-art method of staining.

Protein InstaStain® is a proprietary patented staining method available exclusively from EDVOTEK®.

APPROXIMATE TIME REQUIREMENTS FOR PRE-LAB AND EXPERIMENTAL PROCEDURES

1. Pre-lab preparations will require approximately 20 minutes on the day of the lab.
2. Students will require approximately 15 minutes to heat samples and load the gel. Practice gel loading may require an additional 15 minutes if performed the same day of the lab.
3. Electrophoresis will require approximately 1 to 1.5 hours, depending on the voltage.

PreLab Preparations

**RECONSTITUTION OF LYOPHILIZED PROTEIN SAMPLES
(LYPHOPROTEINS™)**

Each tube contains enough material for loading 6 wells.

1. Add 130 μ l of distilled or deionized water to each tube (A-D) and allow the samples to hydrate for several minutes. Vortex or mix vigorously.
2. Bring a beaker of water, covered with aluminum foil, to a boil. Remove from heat.
3. Make sure the sample tubes A through D are tightly capped and well labeled. The bottom of the tubes should be pushed through the foil and immersed in the boiling water for 5 minutes. The tubes should be kept suspended by the foil.
4. 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 μ l.
5. 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

1. 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 the table below. The buffer should just cover the back plate of the gel cassette.

Tris-Glycine-SDS Electrophoresis (Chamber) Buffer

EDVOTEK Model #	Concentrated Buffer (10x)	+	Distilled Water	=	Total Volume
MV-10	58 ml		522 ml		580 ml
MV-20	95 ml		855 ml		950 ml



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Pre-Lab Preparations

Time and Voltage		
Volts	Recommended Time	
	Minimum	Optimal
125	60 min	75 min

ELECTROPHORESIS TIME AND VOLTAGE

Your time requirements will dictate the voltage and the length of time for your samples to separate by electrophoresis. Approximate recommended times are listed in the table at left.

PREPARING STAINING AND DESTAINING SOLUTIONS

The stock solution is used for staining and destaining with Protein InstaStain®

- Solution for staining with Protein InstaStain®**
 - Prepare a stock solution of Methanol and Glacial Acetic Acid by combining 180 ml Methanol, 140ml Distilled water, and 40ml Glacial Acetic Acid.
 - Staining of Protein Gel(s) is optional.
- Destaining Solution**
 - Use the stock solution of Methanol, Glacial Acetic acid and distilled water (in Step 1) to destain the gel(s).

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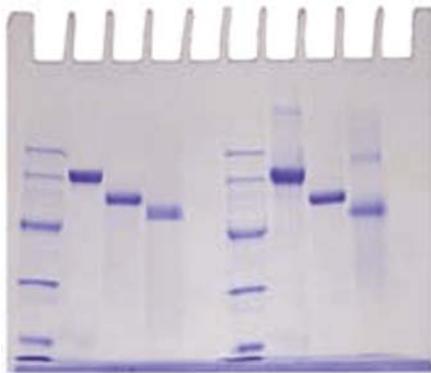
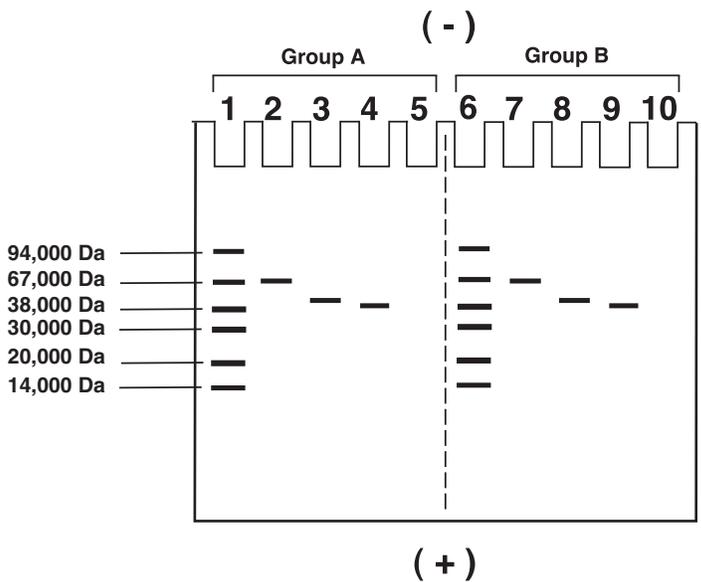
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Idealized Schematic of Results

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	50,000



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Study Questions and Answers

1. The migration rate of glutamate dehydrogenase is very similar to the migration rate of β -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.

Glutamate dehydrogenase is a hexamer with a molecular weight of 300,300 (+/- 2000), β -amylase is a tetramer with a molecular weight of 206,000. Upon denaturation with SDS and 2-mercaptoethanol, 6 subunits of approx. 50,000 will be generated from glutamate dehydrogenase. Likewise, the native β -amylase, which has 4 subunits, will yield a protein band of approx. 50,000

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?

The native molecular weight of a subunit can be determined by this method, however often proteins are aggregates of polypeptide chains. Approximately 1% of protein mass consists of carbohydrate and the structure and mass of the oligosaccharide cannot be anticipated by DNA sequencing. Also, DNA does not yield information on enzyme cofactors.

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.

Disulfide links are still intact. SDS does not cause the cleavage of covalent bonds.

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.

Concentrated solutions of the protein tend to polymerize, forming chains of approximately 6 enzyme molecules. The substrates NADH and glutamate are bound, causing conformational changes in the protein and disaggregation to the 330,000 molecular weight enzyme.

Study Questions and Answers

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.

Carbonic anhydrase has 3 major isoenzymic forms, differing in amino acid sequence and net charge but all having similar molecular weights.

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.

SDS-polyacrylamide gel electrophoresis gives anomalous molecular weight values for proteins having high percentages of carbohydrate. These proteins tend to bind less SDS than normal, which tends to decrease the charge to mass ratio.



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