EDVOTEK®

The Biotechnology Education Company ®

Revised and Updated

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.

Table of Contents

Experiment Components

Upon receipt, freeze Components A-D.

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

		Storage
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

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

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

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.

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

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.

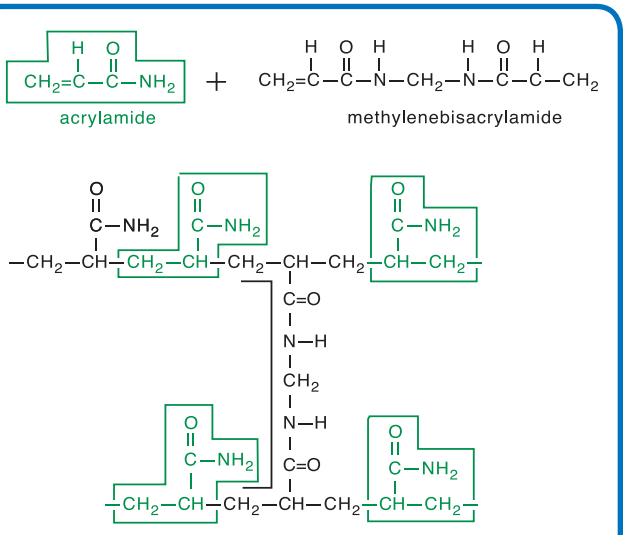


Figure 1:
Schematic of polyacrylamide polymer formation

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).

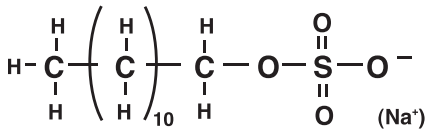


Figure 2:
The chemical structure of sodium dodecylsulfate (SDS).

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.

Background Information

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.

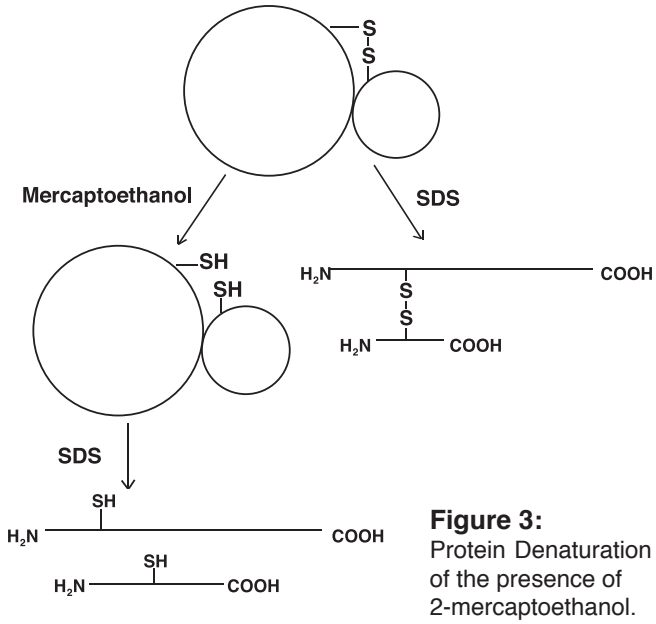


Figure 3: Protein Denaturation of the presence of 2-mercaptoethanol.

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 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.

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

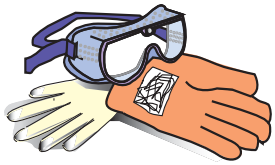
Experiment Procedures

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

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.

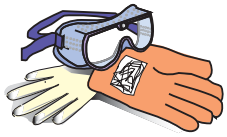
1. Bring a beaker of water, covered with aluminum foil, to a boil. Remove from heat.
2. Make sure the sample tubes A through D are tightly capped and well labeled. The bottom of the tubes should be pushed through the foil and immersed in the boiling water for 5 minutes. The tubes should be kept suspended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

Experiment Procedures



Wear gloves and safety goggles

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.

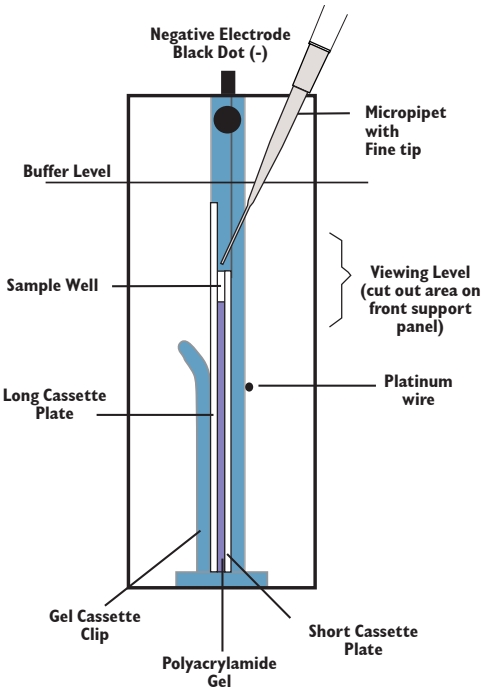
   Note: The front plate is smaller (shorter) than the back plate.

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.

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



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.

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 µl 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

Experiment Procedures

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

Group B

Lane 6	Load 20 µl of Tube A	Standard Protein Markers
Lane 7	Load 20 µl of Tube B	unknown protein 1
Lane 8	Load 20 µl of Tube C	unknown protein 2
Lane 9	Load 20 µl 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

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.*

Experiment Procedures

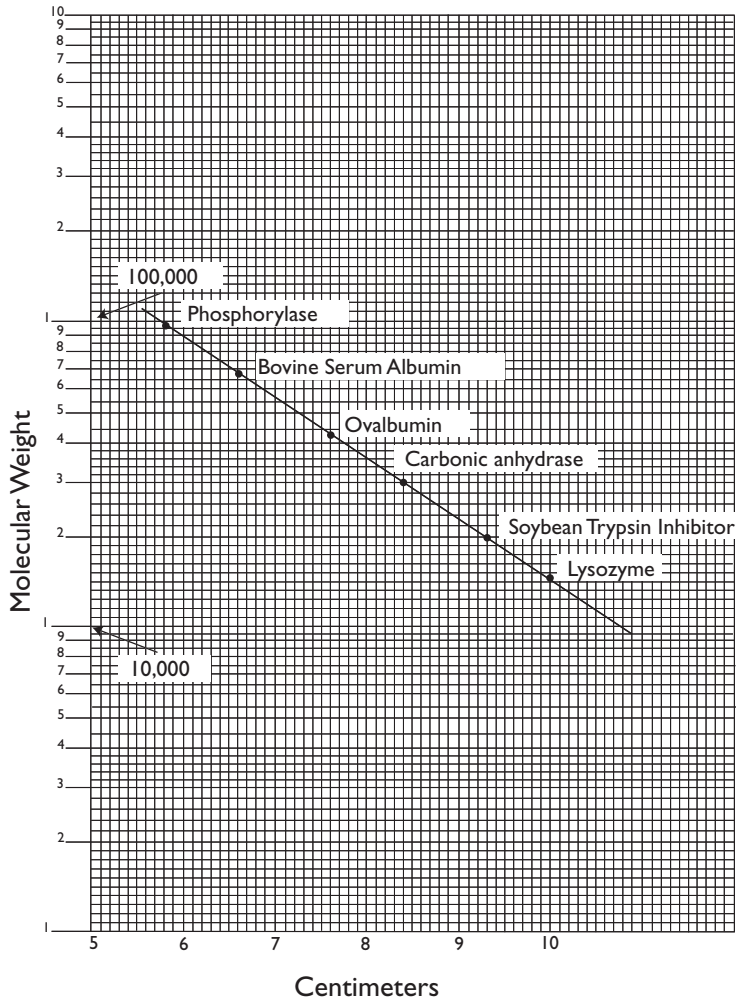
Determination of Molecular Weights

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

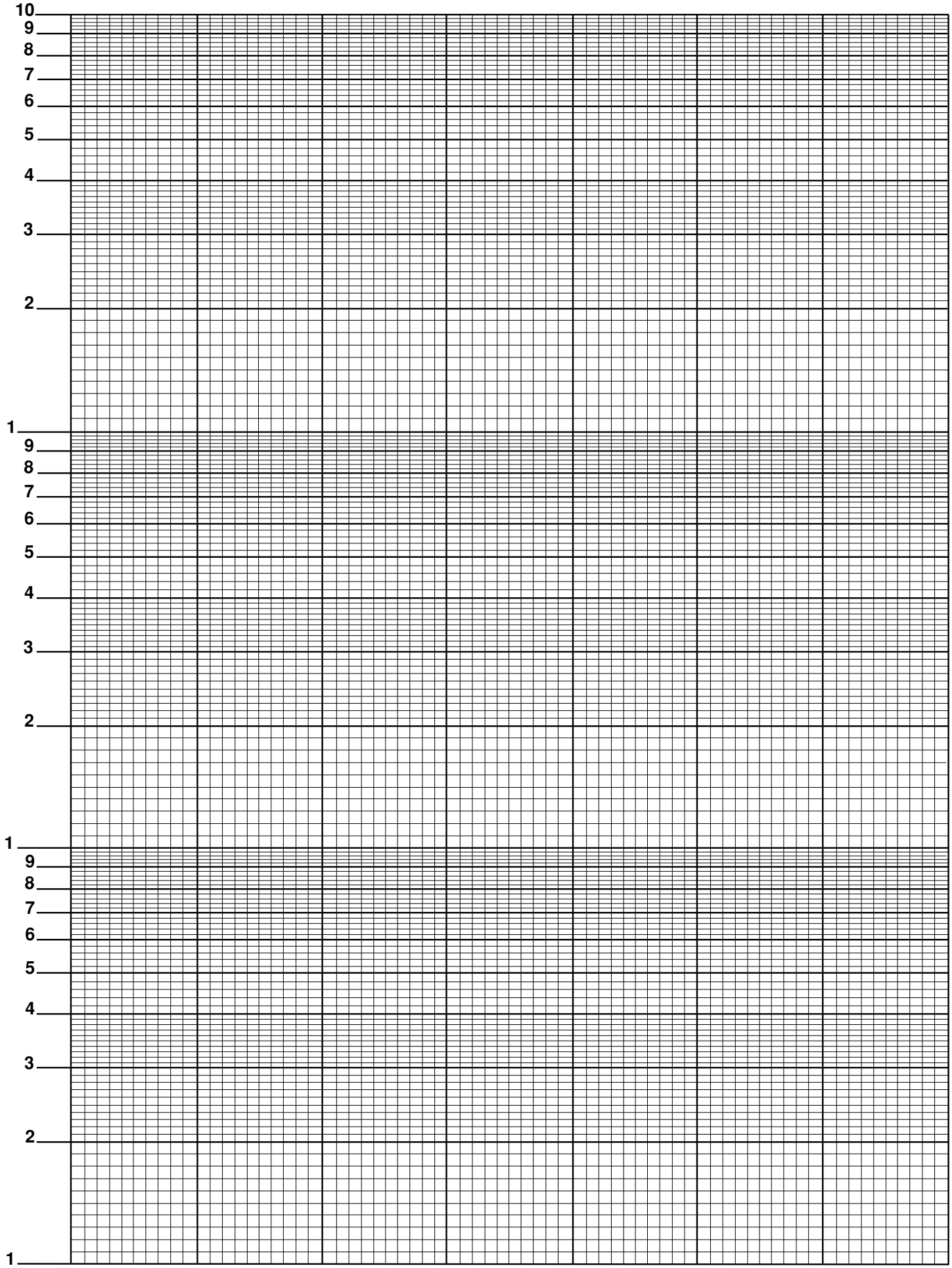
94,000	30,000
67,000	20,000
38,000	14,000

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 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. 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 Rf (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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EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Tris-Glycine SDS Running Buffer (10X)

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **1121 5th St. NW Washington DC 20001**

Emergency Telephone Number: **202.370.1500**

Telephone Number for information: **202.370.1500**

Date Prepared: 5-07-11

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Tris	CAS# 77-86-1			3%
Glycine	CAS# 56-40-6			14%
Sodium dodecyl sulfate	CAS# 151-21-3			1%

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble

Appearance and Odor: Clear, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data		No data	No data

Extinguishing Media: Water spray, carbon dioxide, dry chemical powder or appropriate foam

Special Fire Fighting Procedures: Wear SCBA and protective clothing

Unusual Fire and Explosion Hazards: May emit toxic fumes

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable	X	Strong oxidizing agents, strong acids

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, carbon dioxide, sulfur oxides, sodium oxides

Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry:	Inhalation?	Skin?	Ingestion?
	Yes	Yes	Yes

Health Hazards (Acute and Chronic): May cause irritation to eyes, skin, and mucous membranes.

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
No data	No data	No data	No data

Signs and Symptoms of Exposure: Irritation

Medical Conditions Generally Aggravated by Exposure: Unknown

Emergency First Aid Procedures: Skin/eye contact: flush w/ water. Inhalation: remove to fresh air. Ingestion: Seek medical attention

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear protective clothing. Avoid contact. Mop up with absorbant material and dispose of properly.

Waste Disposal Method: Follow all state, federal, and local regulations.

Precautions to be Taken in Handling and Storing: Avoid contact, keep away from heat.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation	Local Exhaust	No	Special	None	
	Mechanical (General)	Yes	Other	None	

Protective Gloves: Chem resistant — Eye Protection: Safety goggles

Other Protective Clothing or Equipment: Lab coat, coveralls

Work/Hygienic Practices: Prevent contact

EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Protein InstaStain

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **1121 5th St. NW Washington DC 20001**

Emergency Telephone Number: **202.370.1500**

Telephone Number for information: **202.370.1500**

Date Prepared: 05/07/11

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Methanol (Methyl Alcohol) CH_3OH	200ppm	200ppm	No data	90%-100%

Section III - Physical/Chemical Characteristics

Boiling Point	65°C	Specific Gravity (H_2O = 1)	.79
Vapor Pressure (mm Hg.)	96mmHg	Melting Point	N/A
Vapor Density (AIR = 1)	1.11	Evaporation Rate (Butyl Acetate = 1)	4.6

Solubility in Water: Complete (100%)

Appearance and Odor: chemical bound to paper, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
(closed cup) 12°C		6.0%	36%

Extinguishing Media: Use alcohol foam, dry chemical or carbon dioxide. (Water may be ineffective)

Special Fire Fighting Procedures: Wear SCBA with full facepiece operated in positive pressure mode. Move containers from firearea

Unusual Fire and Explosion Hazards: Vapors may flow along surfaces to distant ignition sources. Close containers exposed to heat may explode. Contact w/ strong oxidizers may cause fire.

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable	X	None

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide, Sulfur oxides

Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None

Section VI - Health Hazard Data

Route(s) of Entry:	Inhalation?	Skin?	Ingestion?
	Yes	Yes	Yes

Health Hazards (Acute and Chronic): Irritating to eyes, skin, mucous membranes and upper respiratory tract. Chronic exposure may cause lung damage or pulmonary sensitization

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
	No data	No data	No data

Signs and Symptoms of Exposure: Respiratory tract: burning sensation. Coughing, wheezing, laryngitis, shortness of breath, headache

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: Flush skin/eyes w/ large amounts of water. If inhaled, remove to fresh air. Ingestion: give large amounts of water or milk. Do not induce vomiting.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Evacuate area. Wear SCBA, rubber boots and rubber gloves. Mop up w/ absorptive material and burn in chemical incinerato equipped w/ an afterburner and scrubber.

Waste Disposal Method: Observe all federal, state, and local laws.

Precautions to be Taken in Handling and Storing: Wear protective gear. Avoid contact/inhalation.

Other Precautions: Strong sensitizer

Section VIII - Control Measures

Respiratory Protection (Specify Type): NIOSH/MSHA approved respirator

Ventilation	Local Exhaust	No	Special	Chem fume hood	
	Mechanical (General)	No	Other	None	

Protective Gloves: Rubber — Eye Protection: Splash-proof goggles

Other Protective Clothing or Equipment: Rubber boots

Work/Hygienic Practices: Avoid prolonged or repeated exposure

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EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): **Practice Gel Loading Solution**

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **1121 5th St. NW Washington DC 20001**

Emergency Telephone Number: **202.370.1500**

Telephone Number for information: **202.370.1500**

Date Prepared: 05-07-11

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble

Appearance and Odor: Blue liquid, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data		No data	No data

Extinguishing Media: Dry chemical, carbon dioxide, water spray or foam

Special Fire Fighting Procedures: Use agents suitable for type of surrounding fire. Keep upwind, avoid breathing hazardous sulfur oxides and bromides. Wear SCBA.

Unusual Fire and Explosion Hazards: Unknown

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable	X	None

Incompatibility: None

Hazardous Decomposition or Byproducts: Sulfur oxides, and bromides

Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None

Section VI - Health Hazard Data

Route(s) of Entry:	Inhalation?	Skin?	Ingestion?
	Yes	Yes	Yes

Health Hazards (Acute and Chronic): Acute eye contact: May cause irritation. No data available for other routes.

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
No data available			

Signs and Symptoms of Exposure: May cause skin or eye irritation

Medical Conditions Generally Aggravated by Exposure: None reported

Emergency First Aid Procedures: Treat symptomatically and supportively. Rinse contacted area with copious amounts of water.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear eye and skin protection and mop spill area. Rinse with water.

Waste Disposal Method: Observe all federal, state, and local regulations.

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation	Local Exhaust	Yes	Special	None	
	Mechanical (General)	Yes	Other	None	

Protective Gloves: Yes — Eye Protection: Splash proof goggles

Other Protective Clothing or Equipment: None required

Work/Hygienic Practices: Avoid eye and skin contact