The Biotechnology Education Company ®

EDVO-Kit

110

Molecular Weight Determination of Proteins

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

The objective of this experiment module is to determine the molecular weight of a protein using SDS horizontal gel electrophoresis. Students will develop a basic understanding of protein structure and denaturation.

EDVOTEK, Inc. • 1-800-EDVOTEK • www.edvotek.com

EVT 090703AM

Table of Contents

	Page
Experiment Components	3
Experiment Requirements	3
Background Information	4
Experiment Procedures	
Experiment Overview and General Instructions	7
Preparing for Agarose Gel Electrophoresis	8
Conducting Agarose Gel Electrophoresis	13
Staining the Gel (Optional)	14
Size Determination of DNA Restriction Fragments	15
Study Questions	17
Instructor's Guidelines	
Notes to the Instructor and Pre-Lab Preparations	19
Quick Reference Tables	22
Avoiding Common Pitfalls	23
Experiment Results and Analysis	24
Study Questions and Answers	25
Material Safety Data Sheets	27

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.

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA. None of the experiment components are derived from human sources.

EDVOTEK, The Biotechnology Education Company, and InstaStain are registered trademarks of EDVOTEK, Inc.. Ready-to-Load, Protein Agarose and LyphoProtein are trademarks of EDVOTEK, Inc.

Experiment Components

Quick Reference:

There is enough sample for 6 gels if you are using an automatic micropipet for sample delivery. Use of transfer pipets will yield fewer gels.

Reagent quantities for 6 gels are based upon the use of horizontal gel electrophoresis apparatus, Model #M12, using split trays or both halves of the standard tray.

Although the proteins in this experiment are pre-stained and can easily be visualized directly during and after electrophoresis, staining with EDVOTEK Protein InstaStain® may enhance the visibility of the bands.

READY-TO-LOAD PROTEIN SAMPLES FOR ELECTROPHORESIS

A Pre-stained LyphoProtein™ Gel Marker (Molecular Weight Standard Protein Markers)
B Unknown Pre-stained LyphoProtein™
C Unknown Pre-stained LyphoProtein™
D Unknown Pre-stained LyphoProtein™

Store proteins (A-D) at -20°C.

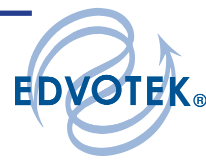
This experiment contains ready-to-load protein samples and reagents sufficient for 6 gels (see Quick Reference).

REAGENTS & SUPPLIES

- Practice Gel Loading Solution
- Protein Agarose™ powder
- Tris-Glycine-SDS electrophoresis buffer (10x)
- 1 ml pipet
- 100 ml graduated cylinder (packaging for samples)
- Microtipped Transfer Pipets
- Semi-log graph paper template
- Protein InstaStain®

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Waterbath
- Recommended equipment:
  - Visualization system (white light)
  - Automatic micropipets with tips
- Pipet pump
- 250ml flasks
- Hot gloves
- Metric rulers
- Distilled or deionized water
- Large beaker
- Methanol (optional)
- Glacial acetic acid (optional)
- Small plastic tray or large weigh boat (optional)



Background Information

Proteins are a highly diversified class of biomolecules. Differences in their chemical properties, such as charge, shape, size and solubility, enables them to perform their individual, unique 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 can be calculated. This assumes the protein does not contain any "non-amino acid" groups (heme, zinc, covalently bonded carbohydrate, etc.) or the amount of these groups, if present, are already known. 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. As an alternative, SDS gel electrophoresis is an easy and inexpensive method commonly used to obtain reliable estimates of protein molecular weights.

A protein can have a net negative or net positive charge, depending on its amino acid composition and specified pH environment. At certain values of pH, the molecule can be overall electrically neutral, i.e. negative and positive charges are balanced. In this case, the protein is isoelectric where the protein will not move in the electric field during electrophoresis. In the presence of an electrical field, a protein with a net charge will migrate towards the electrode of opposite charge.

Proteins exhibit many different three-dimensional shapes and folding patterns which are determined by their amino acid sequence and intracellular processing. The precise three-dimensional configuration of a protein is critical to its function. The general shapes these molecules can have are spherical, elliptical or rod-like. Proteins can consist of a single polypeptide or several polypeptides specifically associated with each other. These polypeptides can be identical, similar or completely different from one another. The number and nature of polypeptides in a protein has large effects on its mass, size and shape. 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 a net charge will migrate through the gel when they are in an electric field. Small molecules pass through gel pores more easily than large ones. Molecules having more of the same charge (positive or negative) than others with the same shape and size will migrate faster. Molecules of the same mass and charge can have different shapes. In this case, those with a more compact shape, like a sphere, will migrate through the gel more rapidly than those with an elongated shape, like a rod. In summary, the amount and charge (net positive or negative), the 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.

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only.
This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc.
Copyright © 1989,1992,1994,1997,1998, 2000, 2004, 2007, 2009, EDVOTEK, Inc., all rights reserved. EVT 090703AM

Background Information

DENATURATION OF PROTEIN STRUCTURES

Sodium dodecylsulfate (SDS) is a detergent which consists of a hydrocarbon chain bonded to a highly negatively charged sulfate group (Figure 1). SDS binds strongly to most proteins and causes them to unfold to a random, rod-like chain. 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 intact polypeptide chains, are called denatured. Proteins which contain several polypeptide chains that are associated only by non-covalent forces will be dissociated by SDS into separate, denatured polypeptide chains. Proteins can contain covalent disulfide crosslinks bonds. These bonds are formed between two cysteine amino acid residues that can be located within the same or different polypeptide chains. High concentrations of reducing agents, such as 2-mercaptoethanol, can break disulfide bonds. This allows SDS to completely dissociate and denature the protein. Figure 2 illustrates a protein containing two different sized polypeptide chains that are cross-linked by a disulfide bond. The chains are also associated by non-covalent forces. The circles represent the native structure.

$$H-CH_2-(CH_2)_{10}-CH_2-O-SO_2-O^- \quad (Na^+)$$

Figure 1:
The chemical structure of sodium dodecylsulfate (SDS).

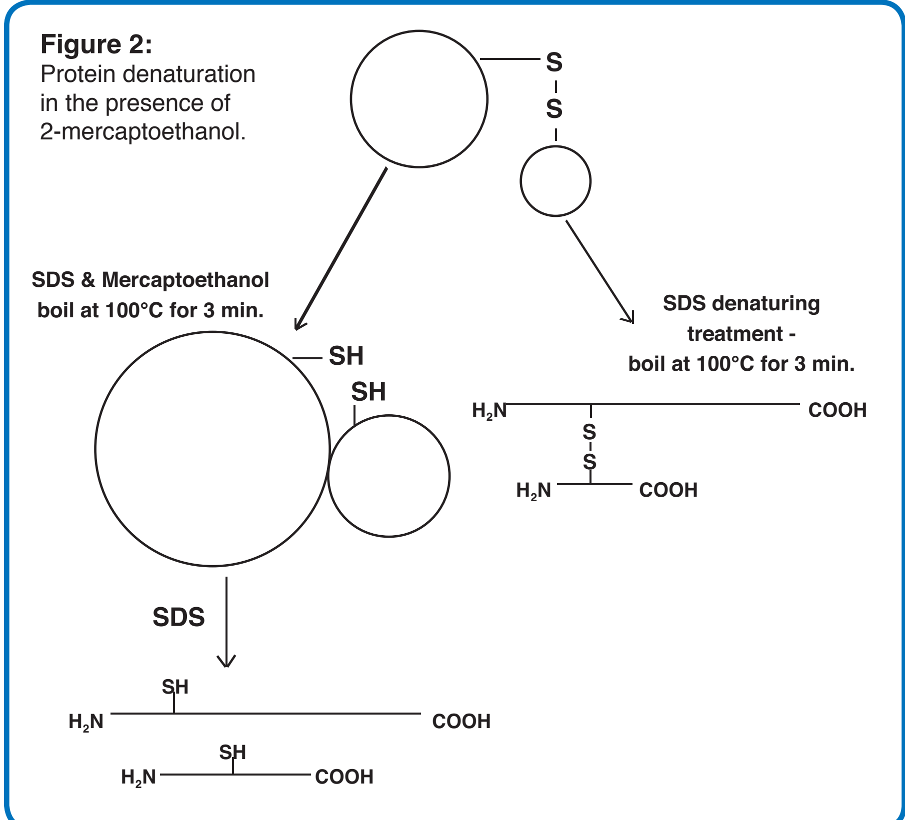


Figure 2:
Protein denaturation in the presence of 2-mercaptoethanol.

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc.
Copyright © 1989,1992,1994,1997,1998, 2000, 2004, 2007, 2009, EDVOTEK, Inc., all rights reserved. EVT 090703AM

Background Information

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 total negative charge of SDS is much more than the intrinsic charges of the protein. SDS efficiently masks these intrinsic charges, consequently, SDS denatured proteins are net negative since the binding of the detergent is proportional to the mass of the protein. The shapes of SDS denatured proteins are the same (rod-like). The larger the molecular weight of the protein, the longer the rod-like chain. During denaturing SDS gel electrophoresis, proteins migrate through the gel towards the positive electrode at a rate that is inversely proportional to their molecular weights. In other words, the smaller the "rod-like" denatured protein, the faster it migrates. The molecular weight of the unknown is obtained by the comparison of its position after electrophoresis to the positions of standard SDS denatured proteins electrophoresed in parallel. The molecular weights of the standard proteins have been previously determined with great accuracy by some of the methods discussed previously. After the proteins have been visualized by staining the gel with dyes, their migration distance can be measured. The log10 of the molecular weights of the standard proteins are plotted versus their migration distance, or RF. Taking the logarithm allows the data to be plotted as a straight line. The molecular weight of the unknown is then easily calculated from the standard curve.

For convenience, the proteins in this experiment have been partially “pre-stained". Protein samples have been covalently conjugated to a blue dye via certain amino acid side chains. This process causes the denaturation of the proteins. However, SDS is still required to produce the electrophoretic mobilities necessary for molecular weight determinations. The mobilities of the conjugates are different from those of the unconjugated forms in the presence of SDS. For this reason, the pre-stained standards cannot be reliably used to determine the molecular weight of unconjugated proteins run in parallel (visualized by staining the gel). The extent of labeling with dye can be variable, producing lot specific variances in apparent pre-stained molecular weights.

The protein standards in this experiment are a mixture having the following denatured molecular weights: 94,000; 67,000; 43,000; 30,000; 20,100. Note that these values are for the dye-conjugated proteins. The molecular weight values have been rounded off for convenience in graphical analysis.

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only.
This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc.
Copyright © 1989,1992,1994,1997,1998, 2000, 2004, 2007, 2009, EDVOTEK, Inc., all rights reserved. EVT 090703AM

Experiment Overview and General Instructions

EXPERIMENT OBJECTIVE:

The objective of this experiment module is to determine the molecular weight of a protein using SDS horizontal gel electrophoresis. Students will develop a basic understanding of protein structure and denaturation.

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

LABORATORY NOTEBOOK RECORDINGS:

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

During the Experiment:

- Record (draw) your observations, or photograph the results.

Following the Experiment:

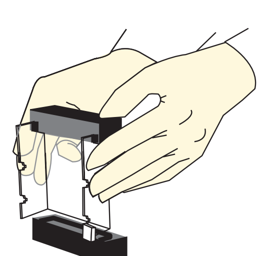
- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only.
This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc.
Copyright © 1989,1992,1994,1997,1998, 2000, 2004, 2007, 2009, EDVOTEK, Inc., all rights reserved. EVT 090703AM

Preparations for Protein Agarose Gel Electrophoresis

PREPARING THE GEL BED

1. Make sure the half gel bed is clean and dry.



2. Close off the open ends of the bed by using rubber dams or tape.

   A. Using Rubber dams:
   - Place a rubber dam on each end of the bed. Make sure the rubber dam sits firmly in contact with the sides and bottom of the bed.

   B. Taping with labeling or masking tape:

   - With 3/4 inch wide tape, extend the tape over the sides and bottom edge of the bed.
   - Fold extended edges of the tape back onto the sides and bottom. Press contact points firmly to form a good seal.

3. Place the well forming template (comb) across the bed in the first set of notches. The comb should sit firmly and evenly across the bed of the half gel.

CASTING THE AGAROSE GEL

This high percent agarose will boil over if not monitored. Keep a close eye on the agarose as it is being melted.

The protein agarose gel concentration required for this experiment is 3.2% weight by volume. Refer to **Table A** for guidelines.

1. Use a 400ml flask to prepare the agarose solution.

   A large beaker is required to prevent the solution from boiling over during heating.

   - Using a beaker, measure and add the distilled water as indicated in **Table A**. Do not add 10x buffer yet at this step. The salts in the buffer tend to cause the agarose solution to boil over.

Table A: Guidelines for Preparing Individual 3.2% Protein Agarose Gels

EDVOTEK Model #	Approximate Gel Bed Dimensions (W x L)	Amt of Protein Agarose	Volume of Distilled Water	Melt agarose in distilled water before adding 10 x buffer.	Volume of Buffer (10x)	Total Volume
M6 or M36	7 x 7 cm	0.96 gm	27 ml		3.0 ml	30 ml
M12	7 x 14 cm	1.92 gm	54 ml		6.0 ml	60 ml

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only.
This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc.
Copyright © 1989,1992,1994,1997,1998, 2000, 2004, 2007, 2009, EDVOTEK, Inc., all rights reserved. EVT 090703AM

Preparations for Protein Agarose Gel Electrophoresis

2. Add the required amount of Protein Agarose powder. Swirl to disperse clumps.

3. With a marking pen, indicate the level of the solution volume on the outside of the flask.

4. Heat the mixture to dissolve the agarose powder. The final solution should appear transparent.

   A. Microwave method:

   - Cover flask with plastic wrap to minimize evaporation.
   - Heat the mixture on High for 1 minute.
   - Gently swirl the mixture and heat on high until all the agarose is dissolved. Use short bursts to avoid boiling over.
   - The mixture should become viscous and transparent with little or no particles of undissolved agarose.

   B. Hot plate or burner method:

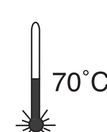
   - Cover the flask with foil to prevent excess evaporation.
   - Place the flask in a large beaker filled with water to avoid burning the slurry by direct exposure to heat.
   - Heat the mixture to boiling over a burner with occasional gentle swirling. Boil until all the agarose is dissolved.

   DO NOT ALLOW AGAROSE TO BOIL OVER.

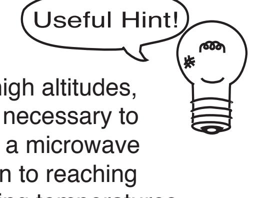
   DO NOT LEAVE THE MIXTURE UNATTENDED DURING HEATING.

   This agarose causes rapid and vigorous boiling. Initially, the mixture will form a large head of fine bubbles that will rise quickly. When this happens, use hot gloves and remove the beaker from the heat. Let the bubbles subside, swirl briefly, and heat the beaker again. Do this several times until the mixture boils evenly.

5. Cool the protein agarose solution to 70°C with occasional gentle swirling to promote even dissipation of heat. If detectable evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.



Wear gloves
and safety goggles



At high altitudes, it is necessary to use a microwave oven to reaching boiling temperatures.

After adding SDS, avoid vigorous swirling to minimize bubbling.

DO NOT POUR BOILING HOT AGAROSE INTO THE BED.
Hot agarose solution may irreversibly warp the bed.

Experiment Procedure

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only.
This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc.
Copyright © 1989,1992,1994,1997,1998, 2000, 2004, 2007, 2009, EDVOTEK, Inc., all rights reserved. EVT 090703AM

Preparations for Protein Agarose Gel Electrophoresis

Experiment Procedure

After the gel is cooled to 70°C:

6. Add the buffer concentrate (10x) as specified in Table A to the cooled agarose solution.

7. Gently swirl to mix. Try to avoid creating excess bubbles.

If using rubber dams, go to step 9. If using tape, continue with step 8.

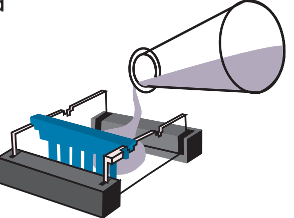
8. Seal the interface of the bed and tape to prevent the agarose solution from leaking.

    - Use a transfer pipet to deposit a small amount of cooled agarose.
    - Wait approximately 2-3 minutes for the agarose to solidify.

9. Pour the cooled agarose solution into the bed. Make sure the bed is on a level surface.

10. Allow the gel to completely solidify. It will become firm and cool to the touch.

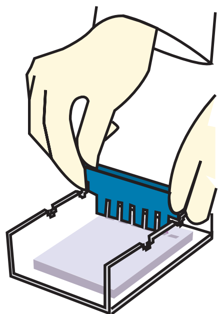
The same 10x concentrated buffer is used for preparing the agarose gel buffer and the chamber buffer. SDS is added to the gel after it is melted.



Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1989,1992,1994,1997,1998, 2000, 2004, 2007, 2009, EDVOTEK, Inc., all rights reserved. EVT 090703AM

Preparations for Protein Agarose Gel Electrophoresis

PREPARING THE SOLIDIFIED GEL FOR ELECTROPHORESIS



1. Carefully remove the rubber dams or tape.
2. Remove the comb by slowly pulling it straight up. Do this carefully and evenly to prevent tearing the sample wells. The comb may be more difficult to remove than lower percentage agarose gels generally prepared for DNA electrophoresis.
3. Inspect the wells by viewing the gel from the edge nearest the wells. If some of the wells are ripped through their bottoms or sides, do not use them when loading samples.
4. Place the gel in the electrophoresis chamber, properly oriented, centered and level on the platform.
5. Fill the chamber of the electrophoresis apparatus with the required volume of diluted buffer as outlined in **Table B**.
6. The gel is now ready for electrophoresis.

NOTE:

This 3.2% Protein Agarose gel is very sturdy and hard.

Table B: Electrophoresis (Chamber) Buffer

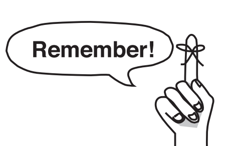
EDVOTEK Model #	Concentrated Buffer (10x)	Distilled Water =	Total Volume
M6 +	30 ml	270 ml	300 ml
M12	40 ml	360 ml	400 ml
M36	100 ml	900 ml	1000 ml

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only.
This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc.
Copyright © 1989,1992,1994,1997,1998, 2000, 2004, 2007, 2009, EDVOTEK, Inc., all rights reserved. EVT 090703AM

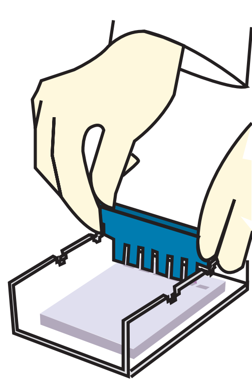
Practice Gel Loading

Quick Reference:

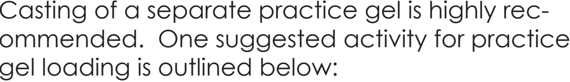
If you are using an automatic micropipet, deliver 20 microliters to the sample well. If using transfer pipets, load the sample well until it is full.



Use plain agar and water to make practice gels - save the agarose for the experiment.



EDVOTEK® experiments which involve electrophoresis contain practice gel loading solution. If your students are unfamiliar with loading samples in agarose gels, it is suggested that they practice sample delivery techniques before performing the electrophoresis part of an experiment. Using the EDVOTEK system, sample delivery can be performed by using either an automatic micropipet, or disposable microtipped transfer pipets.



Casting of a separate practice gel is highly recommended. One suggested activity for practice gel loading is outlined below:

1. Cast a gel with the maximum number of wells and place it under the buffer in an electrophoresis apparatus chamber. (Use plain agar and water to make the practice gels- save the agarose for the experiment.)
2. Let students practice delivering the practice gel loading solution to the sample wells.
3. If students need more practice, remove the practice gel loading solution by squirting buffer into the wells with a transfer pipet.
4. When students are finished practicing, replace the practice gel with a fresh gel and continue with the experiment.

   The practice gel loading solution will become diluted in the buffer and will not interfere with the experiment.

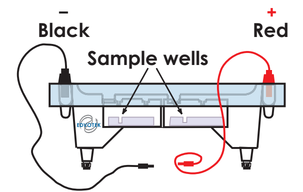
Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1989,1992,1994,1997,1998, 2000, 2004, 2007, 2009, EDVOTEK, Inc., all rights reserved. EVT 090703AM

Conducting Agarose Gel Electrophoresis

This experiment requires a 3.2% Protein Agarose gel. Make sure the electrophoresis apparatus leads reach the power source before loading samples. Do not move the apparatus after the samples are loaded because movement of the unit will cause samples to spill out of the wells.

Reminders:

During electrophoresis, the protein samples migrate through the agarose gel towards the positive electrode. Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.



LOADING PRE-STAINED PROTEIN SAMPLES

1. Bring a beaker of water, covered with aluminum foil, to a boil. Remove from heat.

2. Make sure resuspended tubes are tightly capped and thawed. The bottom of the tubes should be pushed through the foil and immersed in the boiling water for 3-4 minutes. The tubes should be kept suspended by the foil.

3. While the samples are still warm, load 20µl of each sample in tubes A - D into the wells in consecutive order.

RUNNING THE GEL

1. After the samples are loaded, carefully snap the cover down onto the electrode terminals.

   Make sure that the negative and positive indicators on the cover and apparatus chamber are properly oriented.

2. Insert the plug of the black wire into the black input of the power source (negative input). Insert the plug of the red wire into the red input of the power source (positive input).

3. Set the power source at the required voltage and run the electrophoresis for the length of time as determined by your instructor. See **Table C** for time and voltage guidelines.

   When current is flowing properly, you should see bubbles forming on the electrodes.

4. After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads and remove the cover.

5. Transfer the gel to a white light box to enhance visualization of the pre-stained protein bands.

6. If the measurements cannot be done shortly after the electrophoresis is completed, you may wish to stain and/or preserve the gel by performing the steps which follow.

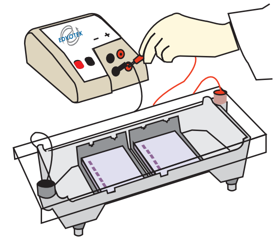


Table C: Time and Voltage

Volts	Recommended Time	
	Minimum	Optimal
125	30 min	45 min
70	40 min	1.5 hrs
50	60 min	2.0 hrs

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only.
This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc.
Copyright © 1989,1992,1994,1997,1998, 2000, 2004, 2007, 2009, EDVOTEK, Inc., all rights reserved. EVT 090703AM

Staining the Gel (Optional)

Although the proteins in this experiment are pre-stained and can easily be visualized directly during and after electrophoresis, staining with EDVOTEK® Protein InstaStain® may enhance the visibility of the bands.

STAINING WITH PROTEIN INSTASTAIN® IN ONE EASY STEP

Protein agarose gels can be stained with Protein InstaStain® cards in one easy step.

1. After electrophoresis, submerge the gel in a small tray with 100 ml of fixative solution. (Use enough solution to cover the gel.)
2. Gently float a sheet of Protein InstaStain® with the stain side (blue) in the liquid. Cover the gel to prevent evaporation.
3. Gently agitate on a rocking platform for 1-3 hours or overnight.
4. After staining, Protein bands will appear as dark blue bands against a light background and will be ready for photography.
   NO DESTAINING IS REQUIRED.
5. If the gel is too dark, destain in several changes of fresh destain solution until the appearance and contrast of the protein bands against the background improves.

Storing the Gel

For long term storage, the gel should be stored in a mixture of 50 ml of distilled water containing 6 ml of acetic acid and 3 ml of glycerol overnight.

Fixative/Destaining Solution for each gel (100ml)

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

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc.
Copyright © 1989,1992,1994,1997,1998, 2000, 2004, 2007, 2009, EDVOTEK, Inc., all rights reserved. EVT 090703AM

Size Determination of DNA Restriction Fragments

The first step for determining the molecular weight of the "unknown" protein polypeptides is to determine the migration distances of the fragments generated after electrophoresis. The molecular weight of the "unknowns" will be extrapolated by graphing their migration distances relative to Sample A, Prestained Lyophilized Protein Gel Markers (Standard markers), for which the molecular weight of each fragment is known.

1. Measure and record the distance traveled in the agarose gel by each Standard marker. In each case, measure from the lower edge of the sample well to the lower end of each band. Record the distance traveled in centimeters (to the nearest millimeter).
2. Label the semi-log graph paper:

   A. Label the non-logarithmic horizontal x-axis "Migration Distance" in centimeters at equal intervals.

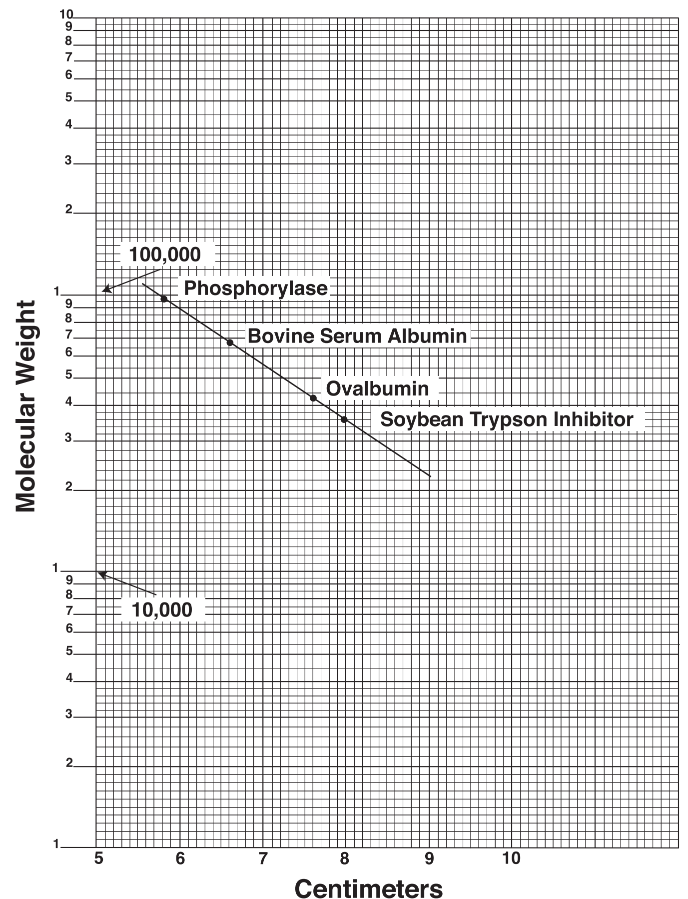
   B. Label the logarithmic vertical y-axis "Molecular Weight".

   Choose your scales so that the data points are well spread out. Assume the first cycle on the y-axis represents 1,000 to 10,000, the second cycle represents 10,000 to 100,000, and the third cycle 100,000 to 1,000,000.

3. For each Standard marker, plot the measured migration distance on the x-axis versus its molecular weight on the y-axis.
4. Draw the best average straight line through all the points.

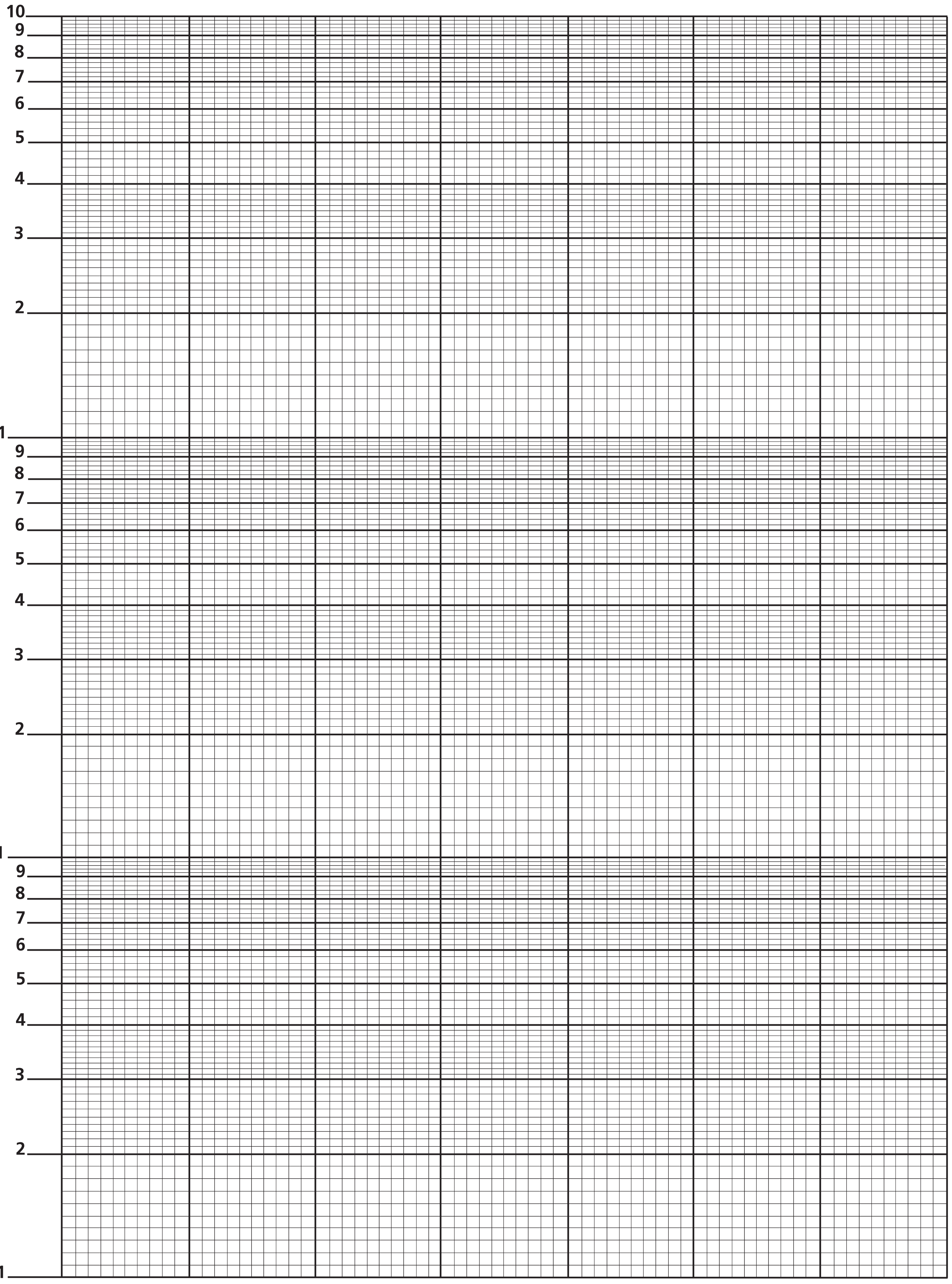
   The line should have approximately equal numbers of points scattered on each side of the line. Some points may be right on the line (see example at left).
5. Measure the migration distance of each of the "unknown" proteins.
6. Using the graph of the Standard markers, determine the molecular weight of each "unknown".
7. Find the migration distance of the unknown protein on the x-axis. Then draw a vertical line from that point until the standard graph line is intersected.
8. From the point of intersection, draw a second line horizontally to the y-axis and determine the apparent molecular weight of the protein polypeptide.

The assignment of molecular weight for proteins separated by agarose gel electrophoresis has a ± 10% margin of error.



Experiment Procedure

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only.
This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc.
Copyright © 1989,1992,1994,1997,1998, 2000, 2004, 2007, 2009, EDVOTEK, Inc., all rights reserved. EVT 090703AM


10
9
8
7
6
5
4
3
2
1
9
8
7
6
5
4
3
2
1
9
8
7
6
5
4
3
2
1

Material Safety Data Sheet

EDVOTEK®

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): Sodium Dodecyl Sulfate (SDS)

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): 14676 Rothgeb Drive, Rockville, MD 20850

Emergency Telephone Number: (301) 251-5990

Telephone Number for information: (301) 251-5990

Date Prepared: 08/04/09

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)
Lauryl Sulfate, Sodium	No data	No data	No data	No data

$C_{12}H_{26}O_4S$
CAS# 151-21-3

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 liquid, 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, alcohol or polymer foam

Special Fire Fighting Procedures: Wear SCBA and protective clothing to prevent contact with skin & eyes.

Unusual Fire and Explosion Hazards: May emit toxic fumes.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		None
	Stable	X	

Incompatibility: Strong oxidizing agents

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

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): May cause irritation to eyes, ears and nose.

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

Signs and Symptoms of Exposure: Respiratory tract: burning sensisation, coughing, wheezing, laryngitis, shortness of breath, & headache

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: Flush skin/eyes with large amounts of water. If inhaled, remove to fresh air.

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 with absorptive material and burn in chemical incinerator equipped with 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 sensitizers

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 googles

Other Protective Clothing or Equipment: Rubber boots

Work/Hygienic Practices: Avoid prolonged or repeated exsposure.

Material Safety Data Sheet

EDVOTEK®

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 Agarose

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): 14676 Rothgeb Drive, Rockville, MD 20850

Emergency Telephone Number: (301) 251-5990

Telephone Number for information: (301) 251-5990

Date Prepared: 08/04/09

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	65°C	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: Souluble-hot, insoluble-cold

Appearance and Odor: White powder, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
Not determined		No data	No data

Extinguishing Media: Water spray, dry chemical, carbon dioxide, halon or standard foam

Special Fire Fighting Procedures: Negligible fire hazard when exposed to heat or flame

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		None
	Stable	X	

Incompatibility: None

Hazardous Decomposition or Byproducts: None hazardous

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): Inhalation: No data available Ingestion: acute large amts cause diarrhea, flatulence & rarelyfecal impaction Skin: no data

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation? None, on FDA list of direct food substances & generally recognized as safe

Signs and Symptoms of Exposure: No data available

Medical Conditions Generally Aggravated by Exposure: No data available except as listed above

Emergency First Aid Procedures: Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Sweep up and place in suitable containers for reclamation or disposal

Waste Disposal Method: Normal solid waste disposal

Precautions to be Taken in Handling and Storing: None

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chem. cartridge respirator with full facepiece, organic vapor cart .

Ventilation				
	Local Exhaust		Special	
	Mechanical (General)	Gen. dilution vent.	Other	

Protective Gloves: Yes; Eye Protection: Splash proof goggles

Other Protective Clothing or Equipment: Impervious clothing to prevent skin contact

Work/Hygienic Practices: None

Material Safety Data Sheets

Full-size (8.5 x 11”) pdf copy of MSDS is available at www. edvotek.com or by request.

Material Safety Data Sheet

EDVOTEK®

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 Buffer

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

Emergency Telephone Number: 301-251-5990

Address (Number, Street, City, State, and ZIP Code): 14676 Rothgeb Drive, Rockville, MD 20850

Telephone Number for information: 301-251-5990

Date Prepared: 08/04/09

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identity Information

Hazardous Components (Specific Chemical Identity; Common Name(s))	OSHA PEL	ACGIH TLV	Other Limits Recommended	% Optional
Lyl Sulfate, Sodium	No data	No data	No data	No data

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 liquid, no odor

Section IV - Fire and Explosion Hazard Data

N.D = No data

Flash Point (Method Used): No data | Flammable Limits | LEL: N.D | UEL: N.D

Extinguishing Media: Water spray, Carbon dioxide, dry chemical powder, alcohol or polymer foam

Special Fire Fighting Procedures: Wear SCBA and protective clothing to prevent contact w/ skin and eyes.

Unusual Fire and Explosion Hazards: May emit toxic fumes.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stability	X	None

Incompatiblity (Materials to avoid): Strong oxidizing agents

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

Hazardous Polymenzation			
	May Occur		
	Will Not Occur	X	None

Section VI - Health Hazard Data

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

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

Carcinogenicity: Not known | NTP? No data | IARC Monographs? No data | OSHA Regulation? No data

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

Medical Conditions Generally Aggravated by Exposure: No data

Emergency and First Aid Procedures: Flush skin/eyes w/ large amounts of water. 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 or spilled: Evacuate area. Wear SCBA, rubber boots and rubber gloves. Mop up w/ absorptive material and burn in chemical incinerator equipped w/ afterburner & scrubber.

Waste Disposal Method: Oserve 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/Hygenic Practices: Avoid prolonged or repeated exposure.

Material Safety Data Sheet

EDVOTEK®

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.

Emergency Telephone Number: (301) 251-5990

Address (Number, Street, City, State, Zip Code): 14676 Rothgeb Drive, Rockville, MD 20850

Telephone Number for information: (301) 251-5990

Date Prepared: 08/04/09

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) CH3OH	200ppm	200ppm	No data	90%-100%

Section III - Physical/Chemical Characteristics

Boiling Point	65°C	Specific Gravity (H_20 = 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): (closed cup) 12°C | Flammable Limits | LEL: 6.0% | UEL: 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.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	None

Incompatibility: Strong oxidizing agents

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

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

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes | Skin? Yes | Ingestion? 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? No data | IARC Monographs? No data | OSHA Regulation? 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

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 Samples

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): **14676 Rothgeb Drive, Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 08/04/09

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)
Lauryl Sulfate, Sodium	No data	No data	No data	No data
$C_{12}H_{26}O_4S$				
CAS # 151-21-3				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_20 = 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 liquid, characteristic, disagreeable 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, alcohol or polymer foam

Special Fire Fighting Procedures: Wear SCBA and protective clothing to prevent contact with skin.

Unusual Fire and Explosion Hazards: May emit toxic fumes

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	None

Incompatibility: Strong oxidizing agents

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

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): May cause irritation to eyes, ears and nose.

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

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 with 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 with absorptive material and burn in chemical incinerator equipped with 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

---

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): **14676 Rothgeb Drive, Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 08/04/09

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_20 = 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			Conditions to Avoid
	Unstable		
	Stable	X	None

Incompatibility: None

Hazardous Decomposition or Byproducts: Sulfur oxides, and bromides

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

Section VI - Health Hazard Data

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

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

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

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