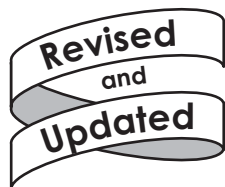




The Biotechnology Education Company®



150
EDVO-Kit #

Survey of Protein Diversity

Storage:

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

EXPERIMENT OBJECTIVES:

The objective of the experiment is to use SDS-polyacrylamide gel electrophoresis to develop an understanding of protein structure, function and diversity.

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.

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

Table of Contents

	Page
Experiment Components	3
Experiment Requirements	3
Background Information	
Survey of Protein Diversity	4
Experiment Procedures	
Experiment Overview	11
Protein Denaturation	12
Electrophoresis of Proteins	13
Staining the Gel	16
Study Questions	18
Instructor's Guidelines	
Notes to the Instructor	19
Pre-Lab Preparations	20
Idealized Schematic of Results	22
Study Questions and Answers	23
Material Safety Data Sheets	24



Experiment Components

Upon receipt,
freeze
Components A-E.

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

- A Standard Protein Markers
- B Milk Whey Proteins
- C Serum Proteins
- D Egg White Proteins
- E Spinach Leaf Proteins

- Tris-glycine-SDS buffer (10x)
- Protein InstaStain®
- Practice gel loading solution
- Transfer pipets

Storage

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

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

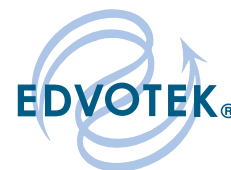
- MV10 or MV20 vertical electrophoresis apparatus
- D.C. power supply
- Three 12% precast SDS polyacrylamide gels (Cat. #651 or #652)
- Micropipet and tips (Cat. #638 Fine Tip Micropipet Tips)
- 500ml graduated cylinder
- Hot plate or burner
- Methanol (150 ml)
- Distilled or deionized water
- Beakers
- 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)

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.

InstaStain, EDVOTEK, and The Biotechnology Education Company are registered trademarks of EDVOTEK, Inc.

LyphoProtein is a trademark of EDVOTEK, Inc.

EDVOTEK • The Biotechnology Education Company®
1.800.EDVOTEK • www.edvotek.com
24-hour FAX: 202.370.1501 • email: info@edvotek.com



Survey of Protein Diversity

Proteins are a highly diversified class of biomolecules. Differences in their chemical properties, such as charge, 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.

Cellular differentiation within an organism is determined by selective expression of genes. Consequently, the kinds of proteins present and their concentrations vary between different tissues. The protein profile within the same tissue can also vary with time and by specific induction of gene transcription with hormones and other chemicals. The majority of cellular proteins remain the same between various mammalian tissue types. The greatest variance in the types of proteins expressed between two different tissues is about 100 fold. However, the concentration of proteins common to different tissues may vary considerably.

The eukaryotic cells of mammals can contain 5,000 to 10,000 different proteins and a total of 5×10^9 protein molecules per cell. The average length of a gene is about 1200 base pairs and the number of base pairs in the haploid human genome is 3×10^9 . Theoretically, 2.4×10^6 different proteins can be encoded. However, less than 1% of the maximum is actually used for this purpose. Many DNA sequences in eukaryotic cells do not code for proteins, such as introns and repetitious sequences, rRNA and tRNA genes, transcriptional regulatory units and pseudogenes. By contrast, *E. coli* contains approximately 2000 different proteins and a total of 3×10^6 protein molecules per cell. There are 4×10^6 base pairs in the *E. coli* genome, theoretically coding for 3,300 different proteins. Over 60% of the genome actually encodes protein. The control of gene expression in prokaryotes is in response to immediate nutritional requirements and adaptations to changes in the physical environment. These features are in accordance with their short life cycle and rapid growth kinetics. The majority of cells in multicellular organisms remain in a stringently regulated constant environment. Gene control in this case deals mainly with differentiation and development, and is generally irreversible. Reversibly inducible genes do occur in higher organisms such as liver cells.

NOTES:

"!" is used to represent factorial.
For example, $n!$ is equal to
 $n(n-1)(n-2)(n-3)\dots(1)$.

Sequence isomerism is the major reason for the great structural and functional diversity of proteins. A tetrapeptide having four different amino acids has $4! = 24$ sequence isomers. A small polypeptide consisting of 20 each of the standard amino acids has $20! = 2 \times 10^{18}$ different sequence isomers. A moderately sized protein of molecular weight 34,000, consisting of only 12 of the standard amino acids, has 10^{300} possible isomers. The sequence variations provide a virtually unlimited set of polypeptides. A protein can have a net negative or a net positive charge, depending on its amino acid composition and the pH. At certain values of pH, the molecule can be electrically neutral overall, i.e. negative and positive charges are balanced. In this case, the protein is isoelectric. In the presence of an electrical field, a protein with a net charge will migrate towards the electrode of opposite charge.



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 © 2005, 2011 EDVOTEK, Inc., all rights reserved. EVT 2011_12_02AM

Survey of Protein Diversity

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. Proteins have spherical, elliptical or rodlike shapes. 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.

POLYACRYLAMIDE GEL COMPOSITION

The properties of proteins affect the way they migrate during gel electrophoresis. Gels used in electrophoresis (e.g. 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 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 charge density and charge, 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 fluids, such as serum.

Sodium dodecylsulfate (SDS) is a detergent which consists of a hydrocarbon chain bonded to a highly negatively charged sulfate group as shown in **Figure 1**. SDS binds strongly to most proteins and causes them to unfold to a random, rodlike 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 their polypeptide chains remaining intact 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 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

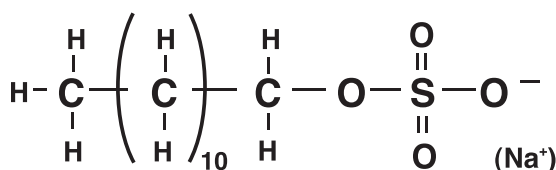
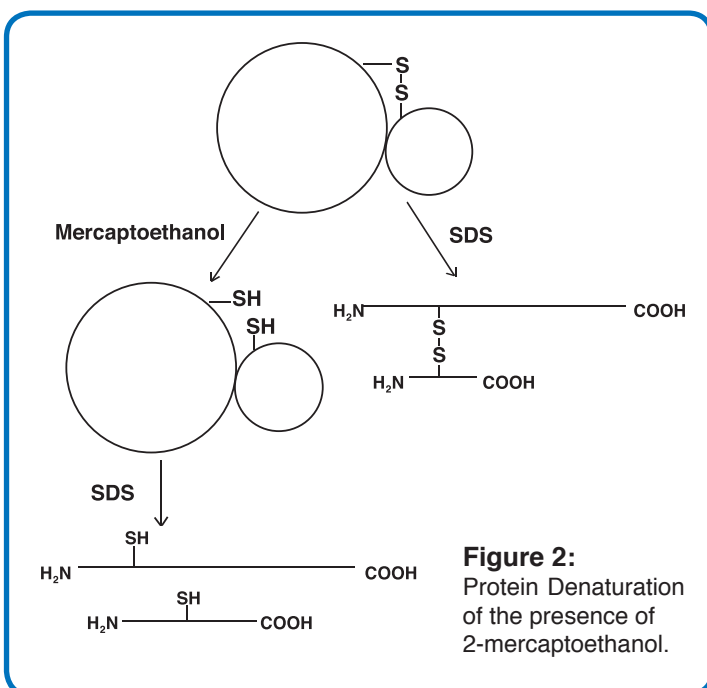


Figure 1:
The chemical structure of sodium dodecylsulfate (SDS).

Survey of Protein Diversity

β -mercaptoethanol, can break disulfide bonds. This allows the SDS to completely dissociate and denature the protein. Proteins that retain their disulfide links bind less SDS, causing anomalous migration. **Figure 2** illustrates these ideas with a protein containing two differently 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.



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 negative charge due to 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. In addition, the shapes of SDS denatured proteins are the same (rodlike). The size of the rodlike chains is the only gross physical difference between SDS denatured proteins. The larger the molecular weight of the protein the longer the rodlike chain. The pores in the gel distinguish these size differences. During SDS electrophoresis, protein migrates through the gel towards the positive electrode at a rate that is inversely proportional to their molecular weight.

In other words, the smaller the protein, the faster it migrates. The molecular weight of the "unknown protein" 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. After the proteins are visualized by staining, 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 or the R_f allows some of the data to be plotted as a straight line. The molecular weight of an unknown protein is then calculated from the standard curve.

The electrophoretic mobility of the proteins is also affected by the gel concentration. Higher percentage gels are more suitable for the separation of smaller proteins and peptides. Polyacrylamide gels are formed by mixing the monomer, acrylamide; the cross-linking agent, methylenebisacrylamide; and



Survey of Protein Diversity

a free radical generator, ammonium persulfate in aqueous buffer (Figure 3). Free radical polymerization of the acrylamide occurs. At various points the acrylamide polymers are bridged to each other by methylenebisacrylamide.

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 usually prepared between two glass plates separated by strips called spacers. As the liquid acrylamide polymerization mixture

is poured between the plates, air is displaced and polymerization proceeds more rapidly.

SURVEY OF PROTEIN SAMPLES

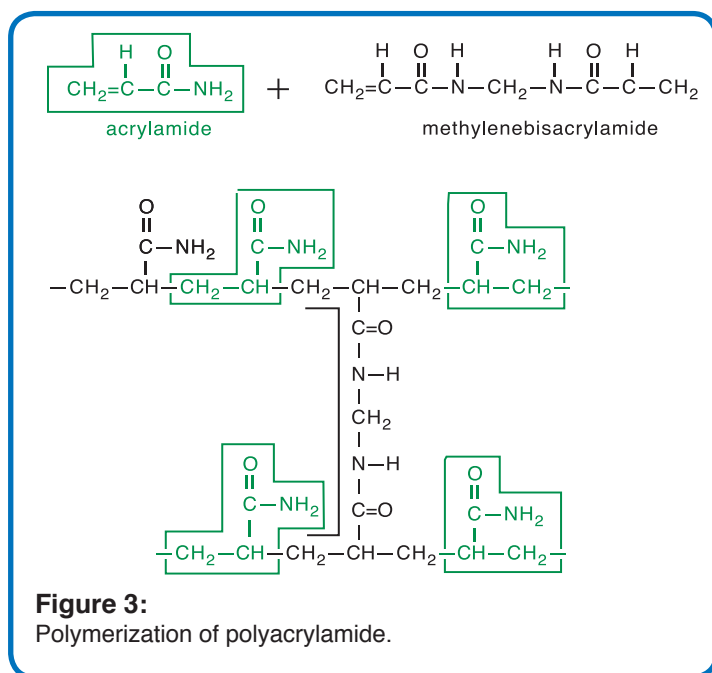
Standard Protein Markers are a mixture of proteins that give the following denatured molecular weights: 94,000; 67,000; 38,000; 30,000; 20,000; and 14,000 Da. The denatured values have been rounded off for convenience in graphical analysis.

Milk Proteins

The major milk proteins are caseins which, with emulsified lipids, gives the liquid its color. In milk the caseins are complexed with calcium which causes them to form aggregates and micelles. The casein fraction can be precipitated from skimmed (defatted) milk by acid titration to pH 4.7. The fat and caseins can also be removed by salting out with ammonium sulfate. The resulting green-yellow supernatant is whey, which contains 20% of the total milk protein.

The whey fraction is a filtered derivative of serum. It contains small amounts of albumin, transferrin and lactoferrin. These proteins may be visualized as faintly staining bands between the 67,000 and 94,000 markers. Transferrin binds and transports iron to the various tissues from the blood plasma and is presumably a source of iron for the nursing young. In the presence of CO_2 transferrin binds 2 Fe^{+3} atoms per molecule. As are most excreted proteins, transferrin is glycosylated and consists of a single polypeptide chain of approximately 80,000 daltons. There are at least 20 different variants of this protein in humans.

Immunoglobulins comprise 10% of the whey proteins. The secretory immunoglobulin IgA predominates. The basic configuration of the immunoglobulins are 2 "light" polypeptide chains of molecular weight 26,000 and



Survey of Protein Diversity

2 "heavy" chains of molecular weight 54,000 to 75,000 depending on the class. Together these chains form a flexible Y-shaped molecule containing numerous intra and interchain disulfide bonds. One disulfide link occurs between each pair of light and heavy chains. Two disulfide links connect the heavy chains with each other. The N-terminal region of both chains are of variable sequence from one molecule to another. Consequently, the immunoglobulin fraction of normal plasma and milk is heterogeneous. The variable regions of both types of chains form the antigen binding site; there are two per molecule. The remaining portions of the light and heavy chains are of constant sequence (within their respective subclasses) from one immunoglobulin molecule to another.

The major whey protein in ruminants is β -lactoglobulin. The native protein has a molecular weight of 35,000 and consists of two identical polypeptide chains. The protein appears as a single band of molecular weight 17,500, just under the 20,000 dalton marker. β -lactoglobulin binds folic acid which is the precursor of tetrahydrofolate, an important coenzyme in metabolic carbon transfer.

α -lactalbumin is a single polypeptide having a molecular weight of approximately 15,000 daltons. It appears as a faint band near the lowest molecular weight marker. This whey protein forms a one-to-one complex with mammary galactosyl transferase. The free enzyme (which occurs in many other tissues) catalyzes the transfer of the galactose unit of UDP-galactose to terminal N-acetylglucosamine residues on the oligosaccharide chains of glycoproteins. When complexed with α -lactalbumin the enzyme substrate specificity is changed to accept glucose as the galactosyl acceptor, resulting in the production of the milk sugar lactose. The α -lactalbumin has no catalytic activity itself but is a specific protein modifier. The galactosyltransferase- α -lactalbumin complex is called lactose synthase.

Blood Serum Proteins

Blood plasma is thought to contain over 100 different proteins. SDS polyacrylamide gel electrophoresis are useful methods for the fractionation and analysis of these proteins, particularly in clinical tests. The SDS electrophoretic profile of the plasma proteins will reveal bands ranging from approximately 200,000 to 15,000 daltons. The largest (top) band in the plasma sample has a denatured molecular weight of 190,000 and corresponds to α_2 -macroglobulin. The native molecular weight of the protein is approximately 800,000. It consists of two dimer subunits associated with each other through non-covalent forces. However, the dimer subunits consist of two polypeptides (190,000) that are associated by non-covalent forces and disulfide bonds. Macroglobulin is a protease inhibitor and may be involved with the control of proteolytic processes such as blood coagulation and complement cascades. Transferrin is a major plasma protein, comprising 3% of the total protein. Transferrin is a major band in the SDS electrophoretic profile, migrating with or just beneath the 94,000 molecular weight marker.



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 © 2005, 2011 EDVOTEK, Inc., all rights reserved. EVT 2011_12_02AM

Survey of Protein Diversity

Background Information

The major band of the plasma proteins is due to albumin, with a molecular weight of approximately 68,000. It is directly under the transferrin band. Albumin is the most abundant plasma protein and is one of the few that is not a glycoprotein. It consists of a single polypeptide chain with 17 inter-chain disulfide bonds. As in the immunoglobulins, the disulfide links help the albumin fold into three structural domains, each consisting of three sub-domains. These domains form the wide variety of ligand binding sites found in the protein. Albumin functions in the binding and transport of fatty acids, Cu^{+2} , Ni^{+2} , bilirubin, tryptophan, steroid hormones, and many drugs such as sulfonamides, penicillin and aspirin. A major physiological role of albumin is in osmotic regulation. The protein is responsible for 80% of the osmotic properties of plasma since it is more than half the plasma proteins by weight, has the lowest molecular weight of the major proteins and contains 18 negative charges at physiological pH. The charge effects the distribution of sodium and chloride ions in the extracellular fluids and consequently, plasma osmolarity. Below the albumin, there are several partially resolved bands that have mobilities between 67,000 and 43,000, as judged by the markers. The heavy chain family of the immunoglobulins (mostly IgG subclasses) migrate in this region in addition to α -antitrypsin (53,000) which is a relatively abundant protein involved with proteolytic inhibition. It consists of 12% carbohydrate by weight. Abnormally low levels of antitrypsin may cause a predisposition to emphysema since lung cells are damaged by proteolysis.

The haptoglobins are multisubunit proteins with native molecular weights ranging from 100,000 to 400,000. The haptoglobins consist of two pairs of different polypeptide chains, α and β , having molecular weight of 9,000 and 43,000 respectively. The polypeptide chains are joined to each other by disulfide bonds and the protein has the subunit stoichiometry $\alpha_2\beta_2$.

The haptoglobins form a 1:1 complex with hemoglobin. The bound hemoglobin cannot be excreted by the kidneys or cause damage to them. The complexes are destroyed by the reticuloendothelial system and the iron of the heme group is salvaged and reused in heme biosynthesis. Plasma hemoglobin arises from the intravascular hemolysis of red blood cells.

Egg White Proteins

The SDS electrophoretic profile of egg white proteins also reveals a high degree of specialization. Egg white proteins are secreted by the cells of the oviduct under hormonal stimulation. The majority of eggwhite protein consists of ovalbumin. The protein consists of a single globular polypeptide chain having a molecular weight of 45,000. Ovalbumin contains a short oligosaccharide covalently linked to an asparagine residue. The oligosaccharide consists of N-acetylglucosamine and mannose. Ovalbumin functions as a storage form of amino acids for the developing embryo. Egg white also contains globulins which are represented by a prominent band corresponding to a molecular weight of approximately 65,000 to 68,000. Very faint bands may be observable above the globulin band at a molecular weight

Survey of Protein Diversity

of near 80,000 corresponding to conalbumin. This protein is the functional equivalent of transferrin. Lysozyme is an enzyme that degrades the polysaccharide chains in bacterial cell walls, predisposing the cell to lysis. The enzyme is a single polypeptide with a molecular weight of approximately 14,500. Egg white contains substantial amounts of this protein which can be observed as a band co-migrating with the lowest molecular weight marker. There are several functional similarities between egg white and milk protein. Both contain iron transport proteins. They have large amounts of “nutritional” proteins (caseins in milk and ovalbumin in egg) and contain antibacterial surveillance (immunoglobulins in milk and lysozyme in egg). These features satisfy several requirements for the developing young.

Plant Proteins

Spinach leaf proteins reveal a complex pattern of bands after electrophoresis, particularly in the lower molecular weight range. Many of these proteins are from the numerous chloroplasts that are found in the leaf tissue of higher plants. A prominent feature is a major band at a molecular weight of approximately 56,000. This band is due to the enzyme ribulose-1, 5-bisphosphate carboxylase. This enzyme catalyzes the addition of CO_2 to the phosphopentose ribulose biphosphate producing two molecules of the phosphotriose 3-phosphoglycerate. This reaction is responsible for the fixation of carbon dioxide in the dark reactions of photosynthetic organisms. The enzyme is the most abundant protein in plants and the biosphere. The enzyme is responsible for the annual incorporation of 5×10^{14} kilograms of carbon dioxide into organic linkage. The carboxylase is present in the stroma of chloroplasts and contains multiple subunits. The 56,000 polypeptide is termed L, and it is encoded by chloroplast DNA. There is also a small subunit with a molecular weight of 14,000, termed S, which is coded in the nucleus. The band containing the S polypeptide co-migrates with the smallest molecular weight marker in the SDS gel. The subunit stoichiometry is L_8S_8 , giving a native molecular weight of 560,000. The active sites are located on the L subunits. The S subunit is thought to regulate the enzymes activity but this has not been clearly demonstrated. Magnesium ion is required for catalytic activity. The carboxylase's activity is regulated by a chloroplast enzyme which catalyzes the addition of carbon dioxide to specific lysine residues in the L subunits. The modification activates the carboxylase.



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 © 2005, 2011 EDVOTEK, Inc., all rights reserved. EVT 2011_12_02AM

Experiment Overview

INTRODUCTION:

In this experiment, the protein samples have been denatured by incubation with the anionic detergent sodium dodecyl sulfate (SDS) and will be submitted to polyacrylamide gel electrophoresis. 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 electrophoresed in parallel. The protein samples contain buffer, SDS, β -mercaptoethanol as a 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 toward the positive electrode (bottom).

The molecular weight estimates obtained from SDS polyacrylamide gel electrophoresis are of denatured proteins. Since proteins often consist of multiple subunits (polypeptide chains) the method can give minimum subunit molecular weights. Incubation of SDS and β -mercaptoethanol causes disruption of cell membranes and lysis. Clarification of the sample is done by centrifugation and the supernatant is used for electrophoresis.



Wear gloves
and safety
goggles

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.

Protein Denaturation

The protein samples were shipped in lyophilized (freeze-dried) form and have been rehydrated by your instructor. These 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 E) 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 13.*

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 E 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.*

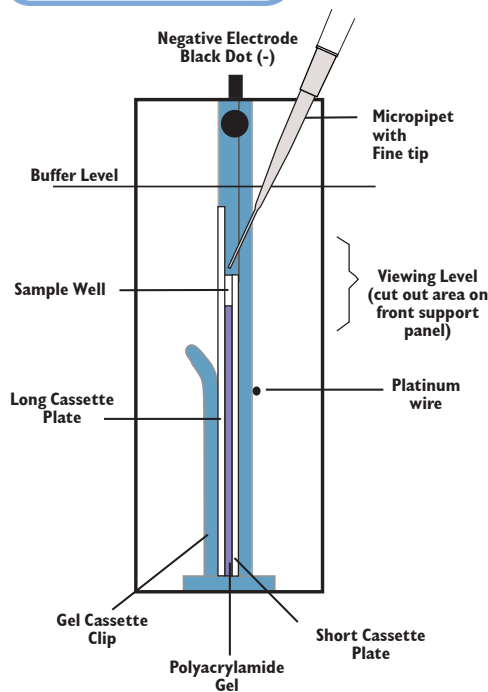


Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 2005, 2011 EDVOTEK, Inc., all rights reserved. EVT 2011_12_02AM

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.

Electrophoresis of Proteins



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 13.

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 samples (20 μ l) 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	milk whey proteins
Lane 3	Load 20 μ l of Tube C	serum proteins
Lane 4	Load 20 μ l of Tube D	egg white proteins
Lane 5	Load 20 μ l of Tube E	spinach leaf proteins

Group B

Lane 6	Load 20 μ l of Tube A	Standard Protein Markers
Lane 7	Load 20 μ l of Tube B	milk whey proteins
Lane 8	Load 20 μ l of Tube C	serum proteins
Lane 9	Load 20 μ l of Tube D	egg white proteins
Lane 10	Load 20 μ l of Tube E	spinach leaf proteins

RUNNING THE GEL

- 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.
- 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).
- 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.
- 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



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.*

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



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 © 2005, 2011 EDVOTEK, Inc., all rights reserved. EVT 2011_12_02AM

Staining the Gel

Storing the Gel

Once satisfactory result is achieved, the gel can be stored in distilled or de-ionized 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.

Ordering Information:

InstaStain® Blue

Cat. #2003 for 40 gels, 7 x 7 cm
Cat. #2004 for 100 gels, 7 x 7 cm
Cat. #2006 Roll, 14 x 350 cm

InstaStain® Ethidium Bromide

Cat. #2001 for 40 gels, 7 x 7 cm
Cat. #2002 for 100 gels, 7 x 7 cm
Cat. #2005 Roll, 14 x 350 cm

InstaStain® Protein

Cat. #2016 for 15 gels, 10 x 10 cm
Cat. #2017 for 30 gels, 10 x 10 cm

Study Questions

1. Would changes occur in the SDS electrophoretic profiles of serum proteins treated in the absence or presence of high concentrations of β -mercaptoethanol? If so, would proteins show differences in migration rates?
2. The α chain of haptoglobin exhibits genetic polymorphism since glutamic acid can be replaced by lysine (a¹⁵ and a^{1F}). Which method could potentially distinguish the polymorphs, SDS or native polyacrylamide gel electrophoresis?
3. An SDS lysate of red blood cells was submitted to SDS polyacrylamide gel electrophoresis. The sample revealed a major band with a molecular weight of approximately 17,000. What protein do you think is responsible for this band?



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 © 2005, 2011 EDVOTEK, Inc., all rights reserved. EVT 2011_12_02AM

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 three major parts:

- 1) separation of proteins on polyacrylamide gels,
- 2) staining of protein bands and
- 3) identifying major protein bands in various extracts.

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®.

Online Ordering
now available



Visit our web site for information about EDVOTEK®'s complete line of experiments for biotechnology and biology education.

Technical Service
Department

Mon - Fri
9:00 am to 6:00 pm ET

FAX: 202.370.1501
web: www.edvotek.com
email: info@edvotek.com

Please have the following information:

- The experiment number and title
- Kit Lot number on box or tube
- The literature version number (in lower right corner)
- Approximate purchase date

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.

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 © 2005, 2011 EDVOTEK, Inc., all rights reserved. EVT 2011_I2_02AM



PreLab Preparations

**RECONSTITUTION OF LYOPHILIZED PROTEIN SAMPLES
(LYPHOPROTEINSM)**

Each tube contains enough material for loading 6 wells.

1. Add 130 μ l of distilled or deionized water to each tube (A-E) 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 E 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



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 © 2005, 2011 EDVOTEK, Inc., all rights reserved. EVT 2011_12_02AM

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

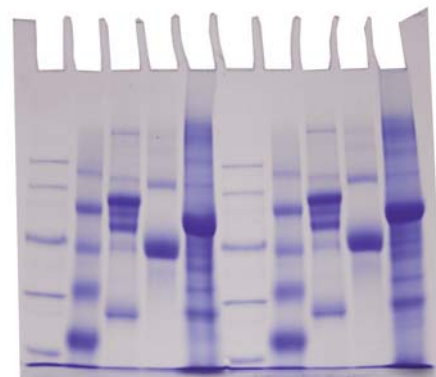
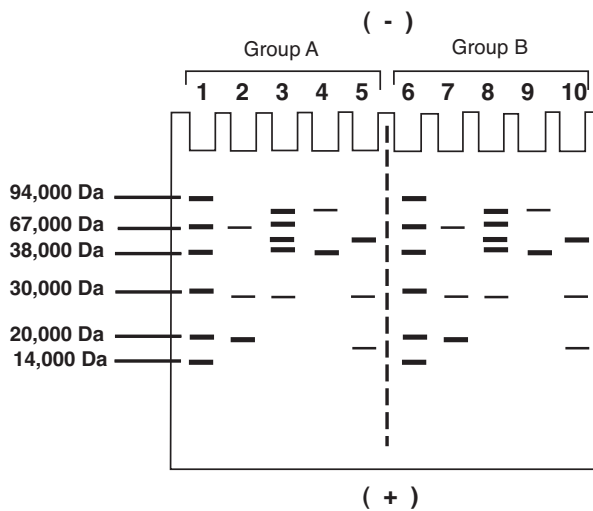
The stock solution is used for staining and destaining with Protein InstaStain®

- 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).

Idealized Schematic of Results


The figure on the left is an idealized schematic showing relative positions of the protein bands. Actual results will yield broader bands of varying intensities. The idealized schematic shows the relative positions of the bands, but are not depicted to scale.


Lanes	Tube	
1, 6	A	Standard Protein Markers
2, 7	B	Milk Whey Proteins
3, 8	C	Serum Proteins
4, 9	D	Egg White Proteins
5, 10	E	Spinach Leaf Proteins




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 © 2005, 2011 EDVOTEK, Inc., all rights reserved. EVT 2011_12_02AM

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

 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) Note: Blank spaces are not permitted. If any item is not applicable, the information is available in the space must be marked to indicate that.	
Section I - Manufacturer's Name: EDVOTEK, Inc. Address (Number, Street, City, State, Zip Code): 1121 5th Street 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 Recommended % (Optional) Other Limits Recommended % (Optional) This CAS# 77-86-1 OSHA PEL ACGIH TLV Recommended % (Optional) Other Limits Recommended % (Optional) Glycine CAS# 56-40-6 3% 14% Sodium dodecyl sulfate CAS# 151-21-3 1% 1%	
Section III - Physical/Chemical Characteristics Boiling Point: No data Specific Gravity (H ₂ O = 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): No data LEL: No data UEL: 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 X Conditions to Avoid: 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 X Conditions to Avoid: None Section VI - Health Hazard Data Routes of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes Health Hazards (Acute and Chronic): May cause irritation to eyes, skin, and mucous membranes. Carcinogenicity: No data NTP? No data IARC Monographs? No data OSHA Regulation? 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 or Spilled: Wear protective clothing. Avoid contact. mop up with absorbent 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): No Other: None Protective Gloves: Chem resistant Eye Protection: Safety goggles Other Protective Clothing or Equipment: Lab coat, coversalls Work/Hygiene Practices: Prevent contact	

 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) Note: Blank spaces are not permitted. If any item is not applicable, the information is available in the space must be marked to indicate that.	
Section I - Manufacturer's Name: EDVOTEK, Inc. Address (Number, Street, City, State, Zip Code): 1121 5th Street 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 Recommended % (Optional) Other Limits Recommended % (Optional) Methylal (Methyl Alcohol) 2300ppm 200ppm No data 90%-100%	
Section III - Physical/Chemical Characteristics Boiling Point: 65°C Specific Gravity (H ₂ O = 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 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. Close containers exposed to heat may explode. Contact w/ strong oxidizers may cause fire.	
Section V - Reactivity Data Stability: Unstable X Conditions to Avoid: None Incompatibility: Strong oxidizing agents Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide, Sulfur oxides Hazardous Polymerization: May Occur X Conditions to Avoid: None Section VI - Health Hazard Data Routes of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes Health Hazards (Acute and Chronic): Irritating to eyes, skin, mucous membranes and upper respiratory tract. May cause lung irritation. May cause irritation to eyes, skin, and mucous membranes. Carcinogenicity: No data NTP? No data IARC Monographs? No data OSHA Regulation? No data Signs and Symptoms of Exposure: Coughing, wheezing, laryngitis, shortness of breath, headache. Medical Conditions Generally Aggravated by Exposure: None Emergency First Aid Procedures: Respiratory tract: burning sensation. Coughing, wheezing, laryngitis, shortness of breath, headache. 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 or Spilled: Wear protective gear. Avoid contact. mop up with absorbent material and ban in chemical laboratories, shops, or maintenance 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: None 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/Hygiene Practices: Avoid prolonged or repeated exposure	

 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) Note: Blank spaces are not permitted. If any item is not applicable, the information is available in the space must be marked to indicate that.	
Section I - Manufacturer's Name: EDVOTEK, Inc. Address (Number, Street, City, State, Zip Code): 1121 5th Street 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 Recommended % (Optional) 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 ₂ O = 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): No data LEL: UEL: 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 X Conditions to Avoid: None Incompatibility: None Hazardous Decomposition or Byproducts: Sulfur oxides, and bromides Hazardous Polymerization: May Occur X Conditions to Avoid: None Section VI - Health Hazard Data Routes 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: NTP? No data 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 or 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/Hygiene Practices: Avoid eye and skin contact	