EDVOTEK®

THE **BIOTECHNOLOGY EDUCATION** COMPANY®

EDVOTEK® AT-HOME: Teacher's Guide

# Survey of Protein Diversity

## Experiment Objective:

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

1.800.EDVOTEK • Fax 202.370.1501 • www.edvotek.com • info@edvotek.com

# Experiment Overview

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.

# Instructions

1. Carefully **READ** the background.
2. **HIGHLIGHT** any words or phrases that you don't know. **LOOK UP** the definition, and **WRITE** the definition into the margins.
3. **UNDERLINE** important information about the experiment.
4. Use this information to **FILL IN** the role of each experimental component.
5. **READ** through the experiment, pausing to **ANSWER QUESTIONS** about what is happening.
6. **ANALYZE** the results.

# Background Information

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 $5 \times 10^9$ 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 $3 \times 10^9$. Theoretically, $2.4 \times 10^6$ 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 $3 \times 10^6$ protein molecules per cell. There are $4 \times 10^6$ 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.

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.

**NOTES:**
**"!"** is used to represent fractorial. For example, n**!** is equal to n (n-1) (n-2) (n-3)...(1).

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.

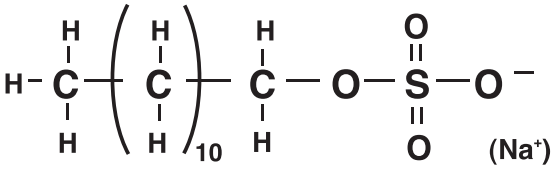



**Figure 1:**
The chemical structure of sodium dodecylsulfate (SDS).

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 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.

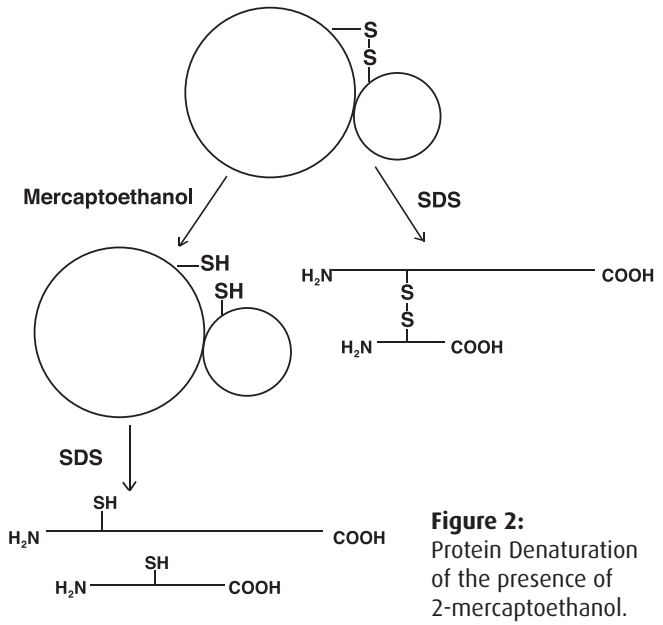



**Figure 2:**
Protein Denaturation of the presence of 2-mercaptoethanol.

In most cases, SDS binds to proteins in a constant ratio of 1.4 grams of SDS per gram of protein. On average, the number of bound SDS molecules is half the number of amino acid residues in the polypeptide. The 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 log10 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.

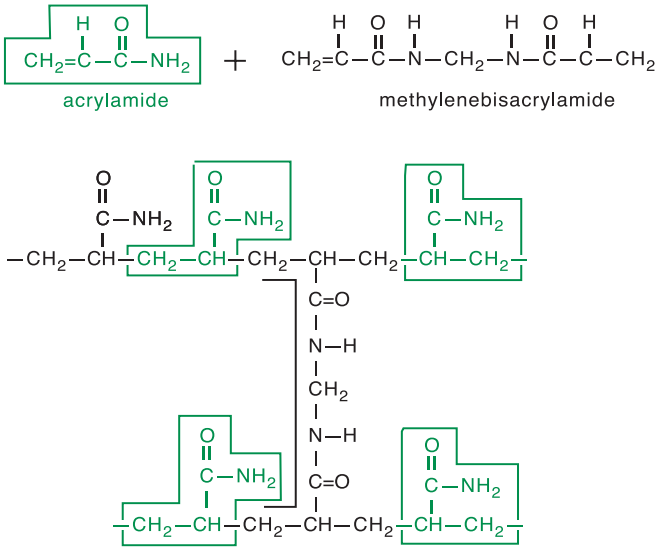



**Figure 3:**
Polymerization of polyacrylamide.

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 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 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 tetrahydrafolate, 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 α2-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.

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 interchain 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 $\alpha$-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, $\alpha$ and $\beta$, 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 $a_2b_2$.

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 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-biphosphate 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 x $10^{14}$ 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.

# 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?**

   Changes would occur since certain multisubunit proteins containing intersubunit disulfide links would not be completely dissociated in the absence of β-mercaptoethanol. Multisubunit proteins would not yield more than one band in the presence of only SDS, if they contain intersubunit disulfide links.

2. **The α chain of haptoglobin exhibits genetic polymorphism since glutamic acid can be replaced by lysine ($a^{1S}$ and $a^{1F}$). Which method could potentially distinguish the polymorphs, SDS or native polyacrylamide gel electrophoresis?**

   Native gel electrophoresis could potentially distinguish $a^{1S}$ and $a^{1F}$ since there is a difference in net charge (glutamate is negative and lysine is positive). SDS gel electrophoresis cannot distinguish the two polymorphs since they are the same size.

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?**

   Hemoglobin is an abundant protein in red blood cells. The protein is a tetramer consisting of 2 α-chains and 2 β-chains of approximately the same molecular weights and subunits of 17,000. The native molecular weight is 68,000.

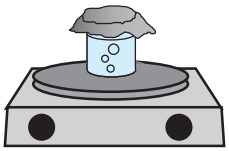
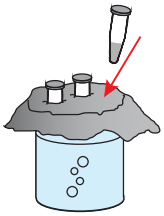
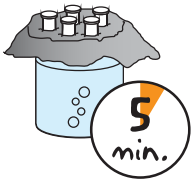
# Experiment Components

Fill in the purpose of each experimental component.

| Component | Purpose |
|---|---|
| • Standard Protein Markers | **Generate standard curve** |
| • Protein Denaturing Solution | **Denatures proteins to single strands** |
| • Tris-Glycine-SDS Electrophoresis Buffer | **Tank buffer to conduct electricty** |
| • Protein Stain | **Allows visualization of proteins** |

# Protein Denaturation

The protein samples 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.

| | | |
|---|---|---|
| 1.  Bring a beaker of water, covered with aluminum foil, to a **BOIL** on a hot plate. **REMOVE** from heat. | 2.  Make sure sample tubes are tightly capped. **PUSH** through the foil and **IMMERSE** in the boiling water. The tubes should be kept suspended by the foil. | 3.   **BOIL** the samples for 5 minutes. STOP **ANSWER QUESTION BELOW.** |
| 4. **REMOVE** the sample tubes from the beaker and allow to **COOL** for a few minutes at room temperature. | | |
| 5. **PROCEED** to loading the gel while samples are still warm. STOP **ANSWER QUESTION BELOW** | | |

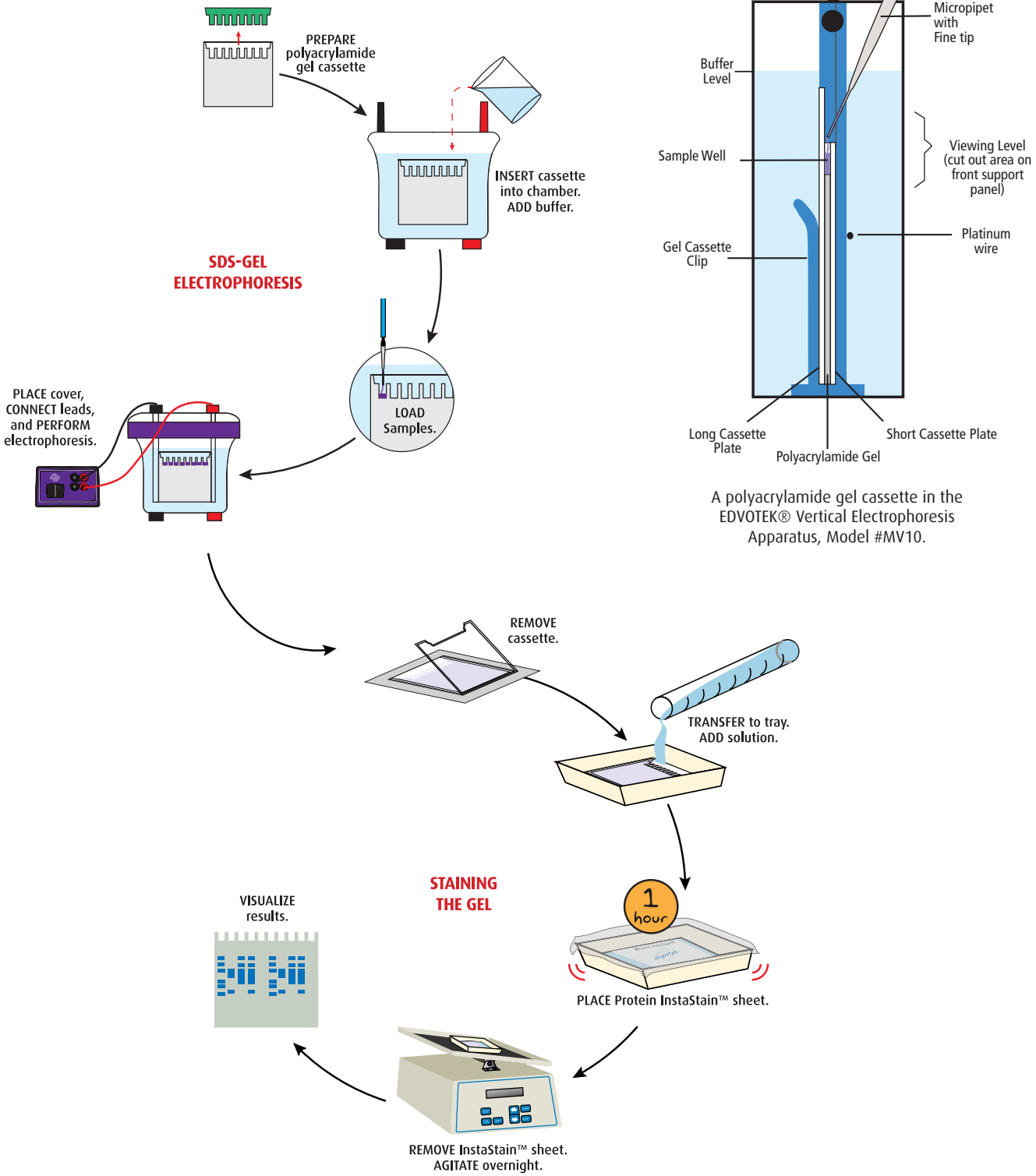
*#3 Q: Why do the proteins need to be boiled before loading?*

**To denature them. Denatured proteins are single-strands and allows them to be separated based on size alone, without deviation due to secondary structure.**

*#5 Q: What would happen if the proteins were loaded while they were cold?*

**The proteins may not be completely denatured, or they may re-nature. If proteins are not denatured, their charge and shape will affect how they run in the gel and the molecular weight will not be accurately determined.**

# Electrophoresis Overview

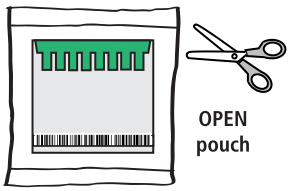



A polyacrylamide gel cassette in the EDVOTEK® Vertical Electrophoresis Apparatus, Model #MV10.

# Electrophoresis of Proteins

## PREPARING PAGE GEL AND CHAMBER

**1.**



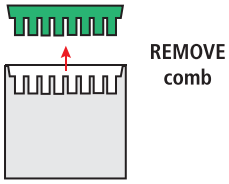

**OPEN** the pouch containing the gel cassette. Remove the cassette and place on bench with the shorter front plate facing up.

STOP **ANSWER QUESTION BELOW.**

**2.**

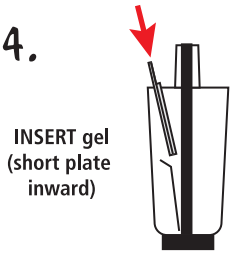

Gels may feature a sticker or tape at the bottom of the front plate. **REMOVE** the tape (if present) to expose the bottom of the gel.

**3.**




Carefully **REMOVE** the comb by gently pulling upwards. Pull the comb straight up to prevent damage to the wells of the gel.

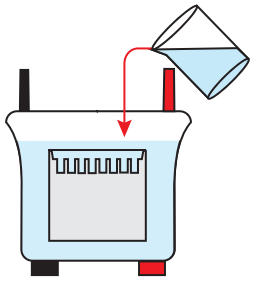
**4.**




**INSERT** the gel into the electrophoresis chamber. Orient the gel according to the manufacturer's instructions.

STOP **ANSWER QUESTION BELOW.**

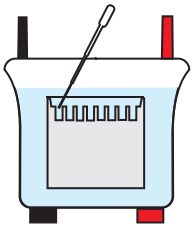
**5.**



**ADD** diluted electrophoresis buffer to the chamber. The buffer should cover the top of the front, shorter plate.

STOP **ANSWER QUESTION BELOW.**

**6.**



**RINSE** each well by squirting electrophoresis buffer into the wells using a transfer pipet. The gel is now ready for sample loading.

*#1 Q: These gels come pre-cast. Use the internet to research polyacrylamide gels and create a hypothesis for why pre-cast gels are sent to classrooms, instead of having students pour their own gels?*

**There are a few possible answers here. One is that polyacrylamide gels consist of both stacking and running gels, making pouring your own gels more difficult. The gels are much thinner, making them more vulnerable to breakage. Also, liquid (non-polymerized) polyacrylamide is a strong neurotoxin, making it unsafe for the classroom.**

*#4 Q: What will happen if the gel is improperly loaded into the unit?*

**The proteins will not migrate correctly in the gel.**

*#5 Q: The electrophoresis buffer is provided as a 10X concentrate. How much water and electrophoresis buffer would you add to make 750 mL of a 1X solution?*

**75 mL of electrophoresis buffer, 675 mL of water.**

# Electrophoresis of Proteins, continued

## LOADING AND RUNNING GEL

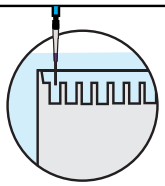
**1.**


Using a fresh fine tip micropipette tip, **MEASURE** 20 µL of the first sample as indicated in Table 1.

Table 1: Gel Loading

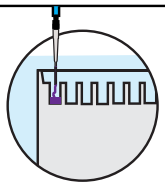
| Lane | Sample |
|---|---|
| 1 | Standard Protein Markers |
| 2 | Milk whey proteins |
| 3 | Serum proteins |
| 4 | Egg white proteins |
| 5 | Spinach leaf proteins |

**2.**



**PLACE** the pipette tip under the buffer, directly above the sample well, resting gently against the back plate of the gel cassette.

**3.**

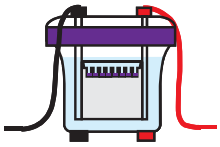


Slowly **DISPENSE** the sample by depressing the plunger.

**4.**

**REPEAT** steps 1-3 for remaining samples in Table 1, changing the tip between each new sample.

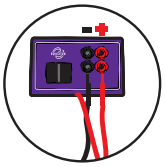
**5.**



Once all samples have been loaded, carefully **PLACE** the cover onto the electrode terminals.

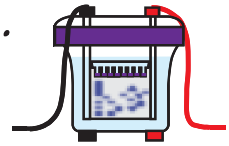
**ANSWER QUESTION BELOW.**

**6.**



**CONNECT** the electrical leads to the power supply, **SET** the voltage and **PERFORM** electrophoresis.

**7.**



**ALLOW** the proteins to separate on the gel for the recommended length of time, or until the tracking dye reaches the bottom of the gel.

**ANSWER QUESTION BELOW.**

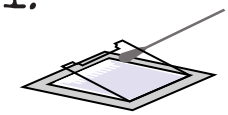
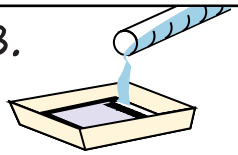
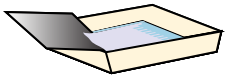
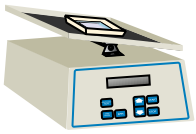
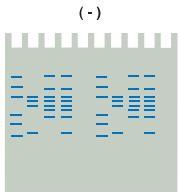
*#5 Q: Why is there SDS in the buffer?*

**SDS is negatively charged and is able to coat the positive amino acids in the proteins, giving the entire sample a negative charge. This allows it to migrate in an analyzable way.**

*#7 Q: How can you tell that the electrophoresis is complete? What will happen if the gel is run for too long?*

**The loading dye will be near or at the end of the gel when the run is complete. If the protein gel is run for too long, then the proteins will run off the gel and into the buffer.**

# Staining the Gel

| | | | |
|---|---|---|---|
| 1.<br>  <br>After electrophoresis, **LAY** the cassette down and **REMOVE** the front plate with a thin spatula. Gently lift it away from the larger back plate. Handle very carefully as the thin gels are extremely fragile. | 2.<br>  <br>**TRANSFER** the gel on the back plate to a clean tray. | 3.<br>  <br>**ADD** a sufficient volume (approximately 100 mL) of the fixative solution into the tray to **COVER** the gel and back plate. (Use enough solution to cover the gel.)<br>STOP **ANSWER QUESTION BELOW.** | 4.<br>  <br>Carefully **REMOVE** the back plate from the tray, leaving just the gel in the tray containing the fixative solution. |
| 5.<br>  <br>**FLOAT** a sheet of Protein InstaStain® with the stain side down in the solution. **COVER** the gel with plastic wrap to prevent evaporation.<br>STOP **ANSWER QUESTION BELOW.** | 6.<br>  <br><br>Allow the Protein InstaStain® paper to **STAIN** the gel for about an hour at room temperature with gentle occasional or continuous agitation. | 7.<br>  <br>**AGITATE** on a rocking platform or just on the lab bench for 2-3 hours. Gels may also be stored overnight if desired. | 8.<br>  <br><br>After staining, Protein bands will appear medium to dark blue against a light background* and will be ready for excellent photographic results. |

*#3 Q: The fixative solution contains methanol and glacial acetic acid. Use the internet to determine what the roles of each of these substances are for fixing proteins.*

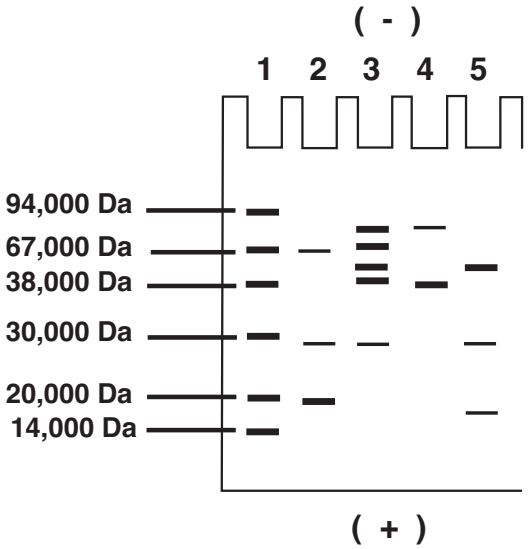
**Fixing proteins means to denature and precipitate them in the gel, which keeps them from diffusing after electrophoresis. Glacial Acetic Acid and Methanol are used for this purpose. They keep the proteins from diffusing and allows the stain to dissolve in solution and bind to the proteins.**

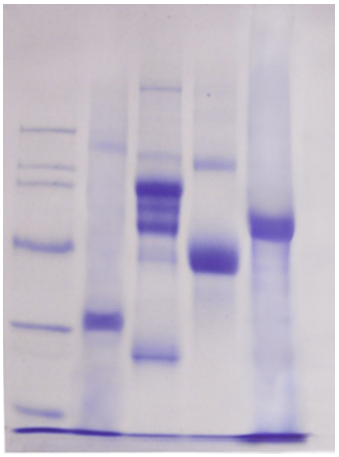
*#5 Q: The InstaStain® card contains coomassie blue. What does coomassie blue do to the gel?*

**Coomassie blue binds to proteins in the gel, dying them a dark blue.**

# 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.



| Lane | Sample |
|---|---|
| 1 | Standard Protein Markers |
| 2 | Milk Whey Proteins |
| 3 | Serum Proteins |
| 4 | Egg White Proteins |
| 5 | Spinach Leaf Proteins |

← Loading Dye Band

## ANALYSIS QUESTIONS

1. Determine the Rf (relative migration distance) of the protein ladder and the strongest band in each lane. The Rf is determined by the equation:

$$Rf = \frac{\text{Migration distance of protein}}{\text{Migration distance of loading dye}}$$

| Band | Rf |
|---|---|
| 94,000 Da | 0.28 |
| 67,000 Da | 0.39 |
| 38,000 Da | 0.44 |
| 30,000 Da | 0.55 |
| 20,000 Da | 0.76 |
| 14,000 Da | 0.96 |
| Milk Whey Protein | 0.76 |
| Serum Protein | 0.46 |
| Egg White Protein | 0.66 |
| Spinach Leaf Protein | 0.53 |

2. Graph the Rf value (x-axis) versus the log10(molecular weight) (y-axis) for the protein ladder to create a standard curve. Use this standard curve to determine the protein size for the major protein of each unknown sample.