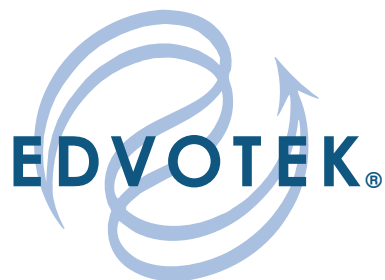


The Biotechnology Education Company®



**252**  
EDVO-Kit #

## Bacterial Protein Fingerprinting

### Storage:

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

### EXPERIMENT OBJECTIVES:

The objective of this experiment is for students to prepare soluble protein lysates from several species of bacteria and analyze the electrophoretic protein profiles to identify an unknown.

All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

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

Upon receipt, refrigerate component E and place G - I in the freezer.

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.

## Experiment Components

A	<i>Escherichia coli</i>	Room temperature
B	<i>Serratia marcescens</i>	Room temperature
C	<i>Micrococcus luteus</i>	Room temperature
D	<i>Bacillus subtilus</i>	Room temperature
E	Tris-EDTA-Glucose (TEG) buffer	Refrigerator
F	Lysozyme	Freezer
G	Protein sample buffer	Freezer
H	Unknown 1 (ready for electrophoresis)	Freezer
I	Unknown 2 (ready for electrophoresis)	Freezer
	• Tris-glycine-SDS electrophoresis buffer (10x)	Room temperature
	• Protein InstaStain®	Room temperature
	• Protein Plus™ stain	Room temperature
	• Practice gel loading solution	Room temperature
	• ReadyPour™ agar	Room temperature
	• Nutrient Broth	Room temperature
	• Screw cap tubes	
	• Sterile inoculating loops	
	• Sterile 1 ml pipets	
	• Sterile petri plates, 100x15 mm	

**None of the components have been prepared from human sources.**

## Requirements

- MV10 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 recommended)
- Microcentrifuge
- Incubation oven
- Microcentrifuge tubes
- Hot plate, Microwave oven or Bunsen burner
- White light box
- Glass staining trays
- Distilled water
- Glacial acetic acid
- 95 - 100% Methanol

## Bacterial Protein Fingerprinting

Bacteria have been historically identified and classified according to morphological traits such as shape, size, motility, gram stain, and macroscopic growth characteristics. However, similar morphological characteristics are shared by many bacteria. Biochemical, nutritional and physiological traits are very important in bacterial identification and classification. These traits include oxygen requirements, glucose fermentation, pH response, ability to grow on selective media, ability to cause hemolysis and response to antibiotics. Bacterial evolutionary descent and phylogenetic classification are tractable by the study of bacterial DNA, RNA and proteins. The approach to bacterial relationships by using molecular phylogenetic data is relatively new. The use of molecular signatures such as ribosomal RNA is useful in the identification of different bacterial species. Bacterial cells, such as *E.coli*, contains approximately 2000 different kinds of proteins. The amount and types of proteins within the cell can vary depending on the environmental and physiological conditions. While many proteins are physico-chemically and functionally similar between different bacteria it is unlikely that all of them will share the same distribution of molecular weights, shapes, charge and immunogenicity. Furthermore, the relative concentrations of proteins that are otherwise similar in other characteristics can vary between two different types of cells and can be used as a fingerprint pattern.

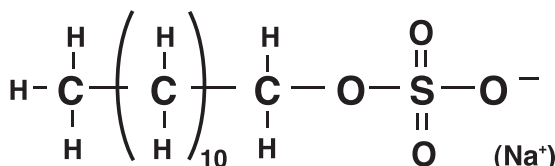


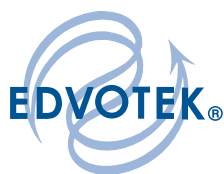
Figure 1 - The chemical structure of sodium dodecylsulfate (SDS).

### ABOUT POLYACRYLAMIDE GEL ELECTROPHORESIS

Sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis is particularly useful to analyzing the complex profile created by a total soluble protein lysate. Total bacterial protein lysates analysis by SDS electrophoresis is frequently done to check for the over expression of protein. Sodium dodecylsulfate (SDS) is a detergent which consists of a hydrocarbon

chain bonded to a highly negatively charged sulfate group (Figure 1).

SDS binds strongly to most proteins and causes them to unfold to a random, rod-like chain. No covalent bonds are broken in this process. Therefore, the amino acid composition and sequence remains the same. Since its specific three-dimensional shape is abolished, the protein no longer possesses biological activity. Proteins that have lost their specific folding patterns and biological activity but have 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 may contain covalent cross-links 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 concentra-



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## Bacterial Protein Fingerprinting

tions of reducing agents, such as 2-mercaptoethanol, will break disulfide bonds. This allows SDS to completely dissociate and denature the protein. Proteins that retain their disulfide links bind less SDS, causing anomalous electrophoretic migration.

In most cases, SDS binds to proteins in a constant ratio of 1.4 grams of SDS per gram of protein. On average, the bound SDS molecules is half the number of amino acid residues in the polypeptide. The large quantity of bound SDS efficiently masks the intrinsic charges in the protein. Consequently SDS denatured proteins are net negative and the binding of the detergent is proportional to the mass of the protein. The charge to mass ratio in denatured proteins is constant and the shapes of SDS denatured proteins are all rodlike. The larger the molecular weight of the protein the longer the rod-like chain. During SDS electrophoresis, proteins migrate through the gel towards the positive electrode at a rate that is inversely proportional to their molecular weight. The pores in the gel distinguish these size differences, the smaller the protein, the faster it migrates. The molecular weights of unknown proteins are obtained by comparison of the relative positions after electrophoresis to the positions of standard SDS denatured proteins electrophoresed in parallel.

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 of peptides. The pore size in polyacrylamide gels is controlled by the gel concentration and the degree of polymer cross linking. The polyacrylamide gel is formed by mixing the monomer, acrylamide, the cross-linking agent, methylenebis-acrylamide, and a free radical generator ammonium persulfate, in aqueous buffer (Figure 2). Free radical polymerization of the acrylamide occurs. At various points the acrylamide polymers are bridged to each other.

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

### SURVEY OF BACTERIAL SAMPLES

*Escherichia coli* and *Serratia marcescens* are gram negative rods of the Enterobacteriaceae family. Members of this family generally can ferment glucose and other sugars, and require minimal media containing salts and small amounts of glucose. These bacteria are facultative anaerobes and do not form spores. Several members such as *E.coli* are found in the intestinal tracts of animals. *S.marcescens* is found in the soil or water. *S.marcescens* produces the red, pyrrole pigment prodigiosin at 25°C in starchy media.

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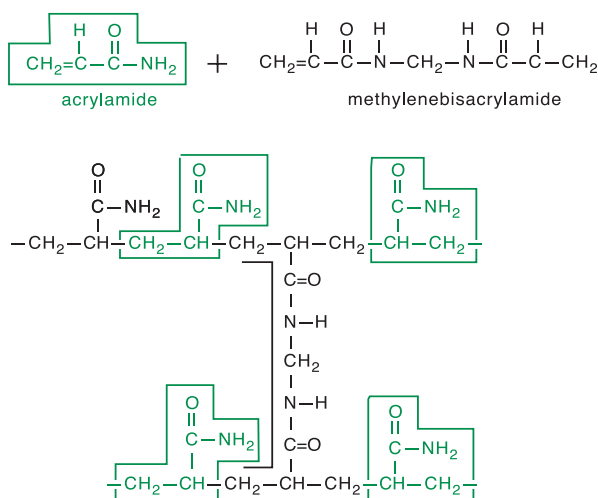
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## Determination of Protein Molecular Weight

*Micrococcus luteus* is gram positive and is a member of the Micrococcaceae family. The Staphylococcus genus is a well known, pathogenic member of this family. The family is characterized by spherically shaped cells that divide to form grape-like clusters. They do not form spores. Members of the genus *Micrococcus* are aerobes and are able to live under a wide variety of conditions in soil, dust, seawater and dairy products. *M.luteus* forms yellow pigments.

*Bacillus subtilis* is a gram positive, rod shaped member of the family Bacillaceae. Members of this family are endospore formers. *B.subtilis* is found in dust and hay. It is a strict aerobe. This bacteria produces the antibiotic subtilin and a closely related strain produces the antibiotic bacitracin. These antibiotics are peptides that interfere with the cell wall synthesis of mostly gram positive bacteria.



**Figure 2:**  
Polymerization of polyacrylamide.

The cell walls of gram negative bacteria consist of an outer lipid membrane containing glycolipids and lipopolysaccharides that project into the external environment. Beneath the outer membrane is the rigid meshwork of peptidoglycan. Beneath this peptidoglycan layer is the periplasmic space which contains proteins. The other side of the space is bounded by the cytoplasmic membrane. The peptidoglycan comprises 5-15% of the cell wall components by weight. The cell walls of gram positive bacteria tend to be much thicker. They do not possess an outer membrane and are structurally simpler. The peptidoglycan can be 20-80% of the cell wall components. The many layers of peptidoglycan in the gram positive bacteria form a relatively homogenous network.

Due to the outer membrane and its molecular projections the gram negative bacteria are much less susceptible to the

activity of lysozyme than the gram positive bacteria. Lysozyme hydrolyzes the glycosidic bonds between the N-acetylglucosamine and N-acetylmuramic acid residues in the peptidoglycan. The viscosity increase is due to the release of high molecular weight DNA and other cell biomolecules. These effects are not readily observed after lysozyme treatment of gram negatives. Conversely, the addition of a detergent such as SDS to a suspension of gram negative cells results in cell lysis as evidenced by increased viscosity. Exposure of gram positive cells with intact peptidoglycan to SDS does not result in significant amounts of lysis as judged by viscosity.

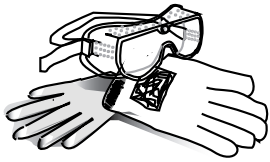


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## Experiment Overview

### EXPERIMENT OBJECTIVE:

The objective of this experiment is for students to prepare soluble protein lysates from several species of bacteria and analyze the electrophoretic protein profiles by identifying the fingerprint pattern of an unknown sample.



Wear gloves  
and safety  
goggles

### LABORATORY SAFETY

Although the bacteria in this experiment are not pathogenic most bacteria are capable of causing infection in certain individuals. Gloves and goggles should be worn. Never mouth pipet. Exercise care when boiling samples. Wear goggles and hot gloves. At the completion of the experiment, all bacterial lysates and plasticware should be sterilized for 20 minutes before disposal. This is common practice when handling materials that have been exposed to bacteria. The tray will prevent liquid from spilling into the sterilizer chamber. Alternatively, the plates and other materials exposed to the cells can be soaked in 10% bleach overnight and then discarded. Wear gloves and goggles when working with bleach.

Un-polymerized acrylamide is a neurotoxin and should be handled with extreme caution in a fume hood. Gloves and goggles must be worn at all times. Use a pipet pump to measure polyacrylamide gel components. Polymerized acrylamide precast gels are safe but should still be handled with gloves.

## Preparation of Bacterial Lysates

In this experiment different species of bacteria will be grown on nutrient agar plates. The cells will be harvested and disrupted under denaturing conditions to obtain a total, crude protein lysate. SDS polyacrylamide gel electrophoresis is capable of resolving hundreds of denaturing protein components on the basis of their size. The bacteria used in this laboratory have characteristic protein electrophoretic profiles. Protein patterns of unknown protein lysates will be compared in parallel with two known samples prepared by the procedures outlined below. After electrophoresis the proteins will be visualized with stain and you will determine whether or not the unknown corresponds to one of the samples you have prepared.

### GROWTH OF BACTERIAL CULTURES

#### First Day

1. Obtain two nutrient agar plates with different letter designations written on the bottom. Your instructor will determine which plates your group will get. Put your lab group number next to the letter on each plate. Each plate will be inoculated with a liquid suspension of the corresponding cells from one of the four bacterial starts identified in Step 4.

#### CHANGE PIPET TIPS BETWEEN EACH PLATE INOCULATION.

2. With a fresh, sterile pipet withdraw 0.1 ml of cell suspension from the lettered vial that corresponds to one of your plates. Deposit the liquid to the surface of the agar at the center of the corresponding plate.
3. Using a sterile loop thoroughly spread the liquid evenly over the entire surface of the agar plate. Rotate the plate and streak back and forth to obtain complete coverage. Do not apply too much pressure otherwise the agar may be damaged. Try to keep the plate partially covered while spreading or work under a flame. Cover the plate after spreading.
4. With a fresh, sterile pipet withdraw 0.1 ml of cell suspension from the lettered vial that corresponds to your second fresh plate. Deposit and spread the liquid as described in steps 2 and 3.

#### Identification

- A - *Escherichia coli*
- B - *Serratia marcescens*
- C - *Micrococcus luteus*
- D - *Bacillus subtilis*

5. Place the plates, INVERTED, in a 37°C incubation oven overnight (15-20 hours).



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## Preparation of Bacterial Lysates

**NOTE:**

*Micrococcus luteus* may grow more slowly than the other microbes. If growth is not evident, continue incubation until a lawn of cells can be seen.

**PREPARATION OF BACTERIAL LYSATES****Second Day**

All the plates should have a confluent mass (lawn) of cells, not colonies. Bacterial lawns may have clumps and aggregates while others may have clearer zones surrounded by heavier growth. Extend the incubation period if sufficient growth is not evident.

6. Label two 10 ml test tubes with the letters corresponding to your plates and your group number. Label two screw cap microcentrifuge tubes with the letters corresponding to your plates and group number. Determine their empty weight to the nearest milligram (0.001g).
7. Add 4 ml of tris-EDTA-glucose (TEG) to each plate.
8. Using a sterile loop for each plate, scrape the cells from the agar surface by streaking back and forth.
9. Tilt the plate slightly. Using a 5 ml pipet with a suction device, pipet the cells up and down to break up larger clumps. Eject the liquid against the tilted agar surface to wash down residual cells. Use a different pipet for each plate.
10. Transfer all the resuspended cells (2.5 to 3.5 ml) from each of the plates to the corresponding 10 ml test tube. Mix the tube by vortexing or by other agitation to break up most of the remaining cell aggregates.
11. Mix the cells to obtain an even suspension. Using fresh pipets with a suction device, transfer 1 ml of the cells to the corresponding screw cap microcentrifuge tubes. Cap each of the tubes.
12. Place your microcentrifuge tubes in the microfuge, counter-balanced and centrifuge for 5 minute at full speed.
13. Remove all of the supernatant from the tubes with a pipet. Discard the supernatants in a waste beaker.
14. Invert the tubes and blot residual liquid with a wipe or paper towel.
15. Determine the wet mass of the cell pellets.

## Preparation of Bacterial Lysates

16. Resuspend the cell pellets with Tris-EDTA-Glucose (TEG) buffer (component E) to approximately 100 mg cells per ml. The volume of buffer to add is given by the cell mass in grams multiplied by 10. For example, if the cell pellet had a mass of 0.025 gm (25 mg), then 0.25 ml of buffer should be added to the tube.

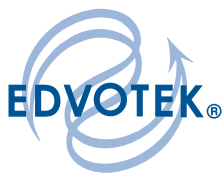
Resuspend the pellet by vigorous vortexing and agitation with a pipet. Mechanically dislodge the pellet under the buffer with a pipet if necessary.

17. Add one-tenth the volume of lysozyme solution to the cell resuspensions (e.g. 25  $\mu$ l to 0.25 ml). Mix. Incubate in a 37°C water bath for 30 minutes.
18. Add three times the volume of protein sample buffer (contains SDS and 2 mercaptoethanol) to the cell suspensions (e.g. 0.75 ml of buffer to 0.25 ml of cells, volume of lysozyme need not be considered).
19. Tighten the screw caps on the tubes. Suspend your sample tubes in a boiling water bath for 10 minutes.
20. Allow the tubes to cool. Centrifuge at full speed, 5 minutes in a microfuge.
21. Transfer most of the supernant to a fresh tube for storage. The supernant is the sample for electrophoresis in the next steps. Pellets may not be clearly visible in the tubes that were centrifuged. Set these tubes aside.



### OPTIONAL STOPPING POINT

The crude protein samples may be kept refrigerated for a week. For longer storage, samples should be frozen but must be boiled again after thawing.



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## Protein Denaturation

Denatured proteins tend to form super-molecular aggregates and insoluble particulates. Heating disrupts aggregates of denatured proteins.

1. Bring a beaker of water, covered with a floating test tube holder or aluminum foil, to a boil. Remove from heat.
2. After proteins have been rehydrated (see PreLab Preparations in Instructor's Guide), 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 10 minutes. The tubes should be kept suspended by the foil.
3. Proceed to loading the gel while the samples are still warm.

### Quick Reference:

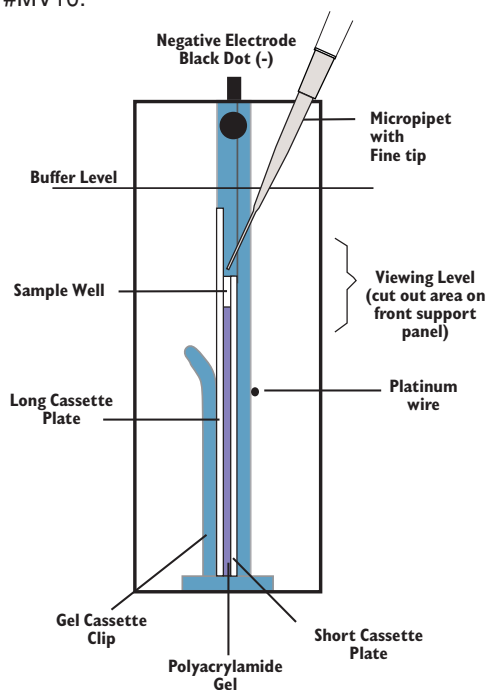
The heating (Steps 1-2) disrupts aggregates of denatured proteins. Denatured proteins tend to form super-molecular aggregates and insoluble particulates.

## Electrophoresis of Proteins



Wear gloves  
and safety goggles

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. Protein samples will not separate in the gel if the cassette is 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 or sample loading.



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## Electrophoresis of Proteins

### READ ME!

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

### PRACTICE GEL LOADING

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

1. Place a fresh fine tip on the micropipet. Aspirate 20  $\mu$ l of practice gel loading solution.
2. Place the lower portion of the fine pipet tip between the two glass plates, below the surface of the electrode buffer, directly over a sample well. The tip should be at an angle pointed towards the well. The tip should be partially against the back plate of the gel cassette but the tip opening should be over the sample well, as illustrated in the figure on page 12.

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 fine 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 protein samples should be loaded in the following manner:

#### Group A

Lane 1	20 $\mu$ l of unknown extract 1 (H)
Lane 2	15 $\mu$ l of <i>E. coli</i> (A)
Lane 3	15 $\mu$ l of <i>S.marcescens</i> (B)
Lane 4	15 $\mu$ l of <i>M.luteus</i> (C)
Lane 5	15 $\mu$ l of <i>B.subtilus</i> (D)

#### Group B

Lane 6	20 $\mu$ l of unknown extract 2 (I)
Lane 7	15 $\mu$ l of <i>E. coli</i> (A)
Lane 8	15 $\mu$ l of <i>S.marcescens</i> (B)
Lane 9	15 $\mu$ l of <i>M.luteus</i> (C)
Lane 10	15 $\mu$ l of <i>B.subtilus</i> (D)

### QUICK REFERENCE:

Protein Samples:

- A. *E.coli*
- B. *S.marascens*
- C. *M.luteus*
- D. *B.subtilus*
- H. Unknown extract 1
- I. Unknown extract 2

### RUNNING THE GEL

- After the samples are loaded, carefully snap the cover all the way down onto the electrode terminals. 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	40 min	1.0 hrs
70	60 min	1.5 hrs



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## Staining the Gel - OPTION #1

### STAINING WITH PROTEIN INSTASTAIN® IN ONE EASY STEP (RECOMMENDED)

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

1. After electrophoresis, carefully open the gel cassette and allow the gel to remain on one of the plates.
2. Submerge the gel and plate in a small tray with 100 ml of fixative solution. (Use enough solution to cover the gel.)
3. Lay the cassette down and remove the front plate by placing a spatula or finger at the top edge, near the sample wells, and lifting it away from the larger back plate. In most cases, the gel will stay on the back plate. If it partially pulls away with the front plate, let it fall onto the back plate.
4. Slide the gel into a staining tray.  
*If the gel sticks to the plate, pipet some of prepared staining solution onto the gel and gently nudge the gel off the plate.*
5. Gently float a sheet of Protein InstaStain® with the stain side (blue) in the liquid. Cover the gel to prevent evaporation.
6. Gently agitate on a rocking platform for 1-3 hours or overnight.
7. After staining, Protein bands will appear medium to dark blue against a light background\* and will be ready for excellent photographic results. **NO DESTAINING IS REQUIRED.**

\*If the gel is too dark, destain in several changes of fresh destain solution until the appearance and contrast of the protein bands against the background improves.

### Storing the Gel

The gel should be stored in a mixture of 50 ml of distilled water containing 6 ml of acetic acid and 3 ml of glycerol overnight.

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.

#### 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

## Staining the Gel - OPTION #2

**STAINING WITH PROTEIN PLUS™ STAIN (LIQUID)**

1. Use a small plastic tray or large weigh boat for staining the gel individually.
2. Remove the gel cassette from the electrophoresis apparatus and blot off excess buffer with a paper towel.
3. Lay the cassette down and remove the front plate by placing a spatula or finger at the top edge, near the sample wells, and lifting it away from the larger back plate. In most cases, the gel will stay on the back plate. If it partially pulls away with the front plate, let it fall onto the back plate.

4. Slide the gel into a staining tray.

*If the gel sticks to the plate, pipet some of prepared staining solution onto the gel and gently nudge the gel off the plate.*

5. Cover the gel with 50 ml of prepared staining solution. The gel should be submerged in stain.
6. Cover the staining tray with plastic wrap to prevent evaporation. Stain for approximately two hours. The gel can be stained in a few hours or overnight but will require a longer destain. (Note: The gel will shrink.)

**Destaining the gel**

7. Pour off the stain from the gel in the staining tray.
8. Add 100 ml of destaining solution. The gel should be submerged under destaining solution.
9. Destain overnight (12 to 14 hours).
10. Remove the gel from the destaining liquid.
11. View the gel on a white light source. Photography of protein bands is optional.
12. Examine the protein bands from the various samples and approximate their molecular weights by comparison with the standard protein markers.

**Storing the Gel**

The gel should be soaked in a mixture of 50 ml of distilled water containing 6 ml of acetic acid and 3 ml of glycerol overnight. The gel can be stored in this solution or dried as described on page 15.

Useful Hint!




Gentle agitation or incubation on a slow rotating or shaking platform will accelerate the destain time.




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## Study Questions


1. Can similar SDS polyacrylamide electrophoretic profiles be definitive criteria for establishing genetic relationships?
2. Are there technical problems you can think of that may cause an incorrect match or a missed match between knowns and unknowns using SDS polyacrylamide gel electrophoresis?
3. Do you know of any electrophoretic methods that could make identification of unknown protein lysates more accurate? (Information is not included in the introduction.)

		<b>Material Safety Data Sheet</b> 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) <b>10x Tris Glycine Buffer</b>		Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.	
<b>Section I</b> Manufacturer's Name <b>EDVOTEK, Inc.</b> Address (Number, Street, City, State, Zip Code) <b>14676 Rothgeb Drive          Rockville, MD 20850</b>		Emergency Telephone Number <b>(301) 251-5990</b> Telephone Number for information <b>(301) 251-5990</b> Date Prepared 09-15-2002 Signature of Preparer (optional)	
<b>Section II - Hazardous Ingredients/Identify Information</b> Hazardous Components [Specific Chemical Identity; Common Name(s)] OSHA PEL ACGIH TLV Other Limits Recommended % (Optional) Glycine (C <sub>2</sub> H <sub>5</sub> NO <sub>2</sub> ) -----No data----- CAS # 56-40-6 Tris CAS # 77-86-1			
<b>Section III - Physical/Chemical Characteristics</b>			
Boiling Point	No data	Specific Gravity (H <sub>2</sub> 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			
<b>Section IV - Physical/Chemical Characteristics</b>			
Flash Point (Method Used)	No data	Flammable Limits	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			


<b>Section V - Reactivity Data</b>			
Stability	Unstable		Conditions to Avoid
	Stable	X	
Incompatibility Strong oxidizing agents			
Hazardous Decomposition or Byproducts Carbon monoxide, carbon dioxide, nitrogen oxides			
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	
<b>Section VI - Health Hazard Data</b>			
Route(s) of Entry:	Inhalation? Yes	Skin? Yes	Ingestion? Yes
Health Hazards (Acute and Chronic) May cause irritation to eyes, skin, and mucous membranes.			
Carcinogenicity:	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			
<b>Section VII - Precautions for Safe Handling and Use</b>			
Steps to be Taken in case Material is Released for Spilled Wear respirator and protective clothing. Mop up with absorbent material and dispose of properly.			
Waste Disposal Method Burn in chemical incinerator equipped w/ afterburner and scrubber. Follow all state, federal, and local regulations.			
Precautions to be Taken in Handling and Storing Avoid contact, keep away from heat.			
Other Precautions None			
<b>Section VIII - Control Measures</b>			
Respiratory Protection (Specify Type)			
Ventilation	Local Exhaust	No	Special None
	Mechanical (General)	Yes	Other None
Protective Gloves	Rubber or PE		Eye Protection Safety goggles
Other Protective Clothing or Equipment Lab coat, coveralls			
Work/Hygienic Practices Prevent contact			

		<b>Material Safety Data Sheet</b> 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) <b>Practice Gel Loading Solution</b>		Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.	
<b>Section I</b> Manufacturer's Name <b>EDVOTEK, Inc.</b> Address (Number, Street, City, State, Zip Code) <b>14676 Rothgeb Drive          Rockville, MD 20850</b>		Emergency Telephone Number <b>(301) 251-5990</b> Telephone Number for information <b>(301) 251-5990</b> Date Prepared 09-19-2002 Signature of Preparer (optional)	
<b>Section II - Hazardous Ingredients/Identify Information</b> Hazardous Components [Specific Chemical Identity; Common Name(s)] OSHA PEL ACGIH TLV Other Limits Recommended % (Optional) This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.			
<b>Section III - Physical/Chemical Characteristics</b>			
Boiling Point	No data	Specific Gravity (H <sub>2</sub> 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			
<b>Section IV - Physical/Chemical Characteristics</b>			
Flash Point (Method Used)	No data	Flammable Limits	LEL No data 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			

<b>Section V - Reactivity Data</b>			
Stability	Unstable		Conditions to Avoid
	Stable	X	None
Incompatibility None			
Hazardous Decomposition or Byproducts Sulfur oxides, and bromides			
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None
<b>Section VI - Health Hazard Data</b>			
Route(s) of Entry:	Inhalation? Yes	Skin? Yes	Ingestion? Yes
Health Hazards (Acute and Chronic) Acute eye contact: May cause irritation. No data available for other routes.			
Carcinogenicity:	NTP? No data available	IARC Monographs? No data available	OSHA Regulation? No data available
Signs and Symptoms of Exposure May cause skin or eye irritation			
Medical Conditions Generally Aggravated by Exposure None reported			
Emergency First Aid Procedures Treat symptomatically and supportively. Rinse contacted area with copious amounts of water.			
<b>Section VII - Precautions for Safe Handling and Use</b>			
Steps to be Taken in case Material is Released for Spilled Wear eye and skin protection and mop spill area. Rinse with water.			
Waste Disposal Method Observe all federal, state, and local regulations.			
Precautions to be Taken in Handling and Storing Avoid eye and skin contact.			
Other Precautions None			
<b>Section VIII - Control Measures</b>			
Respiratory Protection (Specify Type)			
Ventilation	Local Exhaust	Yes	Special None
	Mechanical (General)	Yes	Other None
Protective Gloves	Yes		Eye Protection Splash proof goggles
Other Protective Clothing or Equipment None required			
Work/Hygienic Practices Avoid eye and skin contact			

 <b>Material Safety Data Sheet</b> 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) <b>Protein InstaStain</b>			
Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.			
<b>Section I</b> Manufacturer's Name <b>EDVOTEK, Inc.</b> Address (Number, Street, City, State, Zip Code) <b>14676 Rothgeb Drive            Rockville, MD 20850</b>			
Emergency Telephone Number <b>(301) 251-5990</b>			
Telephone Number for information <b>(301) 251-5990</b>			
Date Prepared 09-19-2002			
Signature of Preparer (optional)			
<b>Section II - Hazardous Ingredients/Identify Information</b>			
Hazardous Components [Specific Chemical Identity; Common Name(s)] OSHA PEL ACGIH TLV Other Limits Recommended % (Optional)			
Methanol (Methyl Alcohol) 200ppm 200ppm No data 90%-100% CH3OH			
<b>Section III - Physical/Chemical Characteristics</b>			
Boiling Point	65°C	Specific Gravity (H <sub>2</sub> 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			
<b>Section IV - Physical/Chemical Characteristics</b>			
Flash Point (Method Used)	Flammable Limits	LEL	UEL
(closed cup) 12°C		6.0%	36%
Extinguishing Media Use alcohol foam, dry chemical or carbon dioxide. (Water may be ineffective)			
Special Fire Fighting Procedures			
Wear SCBA with full facepiece operated in positive pressure mode. Move containers from firearea			
Unusual Fire and Explosion Hazards Vapors may flow along surfaces to distant ignition sources. Close containers exposed to heat may explode. Contact w/ strong oxidizers may cause fire.			

<b>Section V - Reactivity Data</b>			
Stability	Unstable		Conditions to Avoid
	Stable	X	None
Incompatibility Strong oxidizing agents			
Hazardous Decomposition or Byproducts Carbon monoxide, Carbon dioxide, Sulfur oxides			
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None
<b>Section VI - Health Hazard Data</b>			
Route(s) of Entry:	Inhalation?	Skin?	Ingestion?
	Yes	Yes	Yes
Health Hazards (Acute and Chronic) Irritating to eyes, skin, mucous membranes and upper respiratory tract. Chronic exposure may cause lung damage or pulmonary sensitization			
Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?			
	No data	No data	No data
Signs and Symptoms of Exposure Respiratory tract: burning sensation. Coughing, wheezing, laryngitis, shortness of breath, headache			
Medical Conditions Generally Aggravated by Exposure No data			
Emergency First Aid Procedures Flush skin/eyes w/ large amounts of water. If inhaled, remove to fresh air. Ingestion: give large amounts of water or milk. Do not induce vomiting.			
<b>Section VII - Precautions for Safe Handling and Use</b>			
Steps to be Taken in case Material is Released for Spilled Evacuate area. Wear SCBA, rubber boots and rubber gloves. Mop up w/ absorptive material and burn in chemical incinerator equipped w/ an afterburner and scrubber.			
Waste Disposal Method Observe all federal, state, and local laws.			
Precautions to be Taken in Handling and Storing Wear protective gear. Avoid contact/inhalation.			
Other Precautions Strong sensitizer			
<b>Section VIII - Control Measures</b>			
Respiratory Protection (Specify Type) NIOSH/MSHA approved respirator			
Ventilation	Local Exhaust	No	Special Chem fume hood
	Mechanical (General)	No	Other None
Protective Gloves	Rubber	Eye Protection	Splash-proof goggles
Other Protective Clothing or Equipment Rubber boots			
Work/Hygienic Practices Avoid prolonged or repeated exposure			

 <b>Material Safety Data Sheet</b> 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) <b>Protein Plus Stain</b>			
Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.			
<b>Section I</b> Manufacturer's Name <b>EDVOTEK, Inc.</b> Address (Number, Street, City, State, Zip Code) <b>14676 Rothgeb Drive            Rockville, MD 20850</b>			
Emergency Telephone Number <b>(301) 251-5990</b>			
Telephone Number for information <b>(301) 251-5990</b>			
Date Prepared 09-19-2002			
Signature of Preparer (optional)			
<b>Section II - Hazardous Ingredients/Identify Information</b>			
Hazardous Components [Specific Chemical Identity; Common Name(s)] OSHA PEL ACGIH TLV Other Limits Recommended % (Optional)			
Methanol (Methyl Alcohol) 200ppm 200ppm No data 90%-100% CH3OH			
<b>Section III - Physical/Chemical Characteristics</b>			
Boiling Point	65°C	Specific Gravity (H <sub>2</sub> 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 Blue liquid/alcoholic, pungent odor			
<b>Section IV - Physical/Chemical Characteristics</b>			
Flash Point (Method Used)	Flammable Limits	LEL	UEL
(closed cup) 12°C		6.0%	36%
Extinguishing Media Use alcohol foam, dry chemical or carbon dioxide. (Water may be ineffective)			
Special Fire Fighting Procedures			
Wear SCBA with full facepiece operated in positive pressure mode. Move containers from firearea			
Unusual Fire and Explosion Hazards Vapors may flow along surfaces to distant ignition sources. Close containers exposed to heat may explode. Contact w/ strong oxidizers may cause fire.			

<b>Section V - Reactivity Data</b>			
Stability	Unstable		Conditions to Avoid
	Stable	X	None
Incompatibility Strong oxidizing agents			
Hazardous Decomposition or Byproducts Carbon monoxide, Carbon dioxide, Sulfur oxides			
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None
<b>Section VI - Health Hazard Data</b>			
Route(s) of Entry:	Inhalation?	Skin?	Ingestion?
	Yes	Yes	Yes
Health Hazards (Acute and Chronic) Irritating to eyes, skin, mucous membranes and upper respiratory tract. Chronic exposure may cause lung damage or pulmonary sensitization			
Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?			
	No data	No data	No data
Signs and Symptoms of Exposure Respiratory tract: burning sensation. Coughing, wheezing, laryngitis, shortness of breath, headache			
Medical Conditions Generally Aggravated by Exposure No data			
Emergency First Aid Procedures Flush skin/eyes w/ large amounts of water. If inhaled, remove to fresh air. Ingestion: give large amounts of water or milk. Do not induce vomiting.			
<b>Section VII - Precautions for Safe Handling and Use</b>			
Steps to be Taken in case Material is Released for Spilled Evacuate area. Wear SCBA, rubber boots and rubber gloves. Mop up w/ absorptive material and burn in chemical incinerator equipped w/ an afterburner and scrubber.			
Waste Disposal Method Observe all federal, state, and local laws.			
Precautions to be Taken in Handling and Storing Wear protective gear. Avoid contact/inhalation.			
Other Precautions Strong sensitizer			
<b>Section VIII - Control Measures</b>			
Respiratory Protection (Specify Type) NIOSH/MSHA approved respirator			
Ventilation	Local Exhaust	No	Special Chem fume hood
	Mechanical (General)	No	Other None
Protective Gloves	Rubber	Eye Protection	Splash-proof goggles
Other Protective Clothing or Equipment Rubber boots			
Work/Hygienic Practices Avoid prolonged or repeated exposure			

**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) Lysozyme		Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.	
<b>Section I</b>			
Manufacturer's Name <b>EDVOTEK, Inc.</b>		Emergency Telephone Number <b>(301) 251-5990</b>	
Address (Number, Street, City, State, Zip Code) <b>14676 Rothgeb Drive Rockville, MD 20850</b>		Telephone Number for information <b>(301) 251-5990</b>	
		Date Prepared <b>09-18-2002</b>	
		Signature of Preparer (optional)	
<b>Section II - Hazardous Ingredients/Identify Information</b>			
Hazardous Components [Specific Chemical Identity; Common Name(s)] OSHA PEL ACGIH TLV Other Limits Recommended % (Optional)			
Lysozyme -----No data-----			
CAS # 12650-88-3			
<b>Section III - Physical/Chemical Characteristics</b>			
Boiling Point	No data	Specific Gravity (H <sub>2</sub> 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 liquid, no odor	
<b>Section IV - Physical/Chemical Characteristics</b>			
Flash Point (Method Used)	No data	Flammable Limits	LEL No data UEL No data
Extinguishing Media Water spray, carbon dioxide, dry chemical powder, alcohol or polymer foam			
Special Fire Fighting Procedures Wear SCBA and protective clothing to prevent contact w/ skin and eyes.			
Unusual Fire and Explosion Hazards None			

<b>Section V - Reactivity Data</b>			
Stability	Unstable		Conditions to Avoid
	Stable	X	No data
Incompatibility		None	
Hazardous Decomposition or Byproducts No data			
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	No data
<b>Section VI - Health Hazard Data</b>			
Route(s) of Entry:	Inhalation?	Skin?	Ingestion?
	Yes	Yes	Yes
Health Hazards (Acute and Chronic) Prolonged or repeated exposure may cause allergic reactions in sensitive individuals			
Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
	None	No data	No data
Signs and Symptoms of Exposure Allergic reactions			
Medical Conditions Generally Aggravated by Exposure No data			
Emergency First Aid Procedures Ingestion: Rinse mouth w/ water, eye/skin contact: flush w/ large amounts of water Inhalation: remove to fresh air			
<b>Section VII - Precautions for Safe Handling and Use</b>			
Steps to be Taken in case Material is Released for Spilled Wear respirator, chemical safety goggles, rubber boots and heavy rubber gloves. Sweep up and place in bag for disposal.			
Waste Disposal Method Dissolve or mix w/ combustible solvent and burn in a chemical incinerator equipped w/ an afterburner and scrubber. Observe federal, state, and local regulations.			
Precautions to be Taken in Handling and Storing Wear protective clothing, avoid contact			
Other Precautions None			
<b>Section VIII - Control Measures</b>			
Respiratory Protection (Specify Type)			
Ventilation	Local Exhaust	No	Special None
	Mechanical (General)	Yes	Other None
Protective Gloves	Chem. resistant gloves	Eye Protection Chem safety goggles	
Other Protective Clothing or Equipment		Lab coat	
Work/Hygienic Practices		Avoid contact	