EDVOTEK®

The Biotechnology Education Company ®

Revised and Updated

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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.

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EVT 2011_12_02AM

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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 F - H 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.

ReadyPour is a trademark and InstaStain, EDVOTEK, and The Biotechnology Education Company are registered trademarks of EDVOTEK, Inc.

EXPERIMENT COMPONENTS

		Storage
A	*Escherichia coli* slant	Room temperature
B	*Serratia marcescens* slant	Room temperature
C	*Micrococcus luteus* slant	Room temperature
D	*Bacillus subtilus* slant	Room temperature
E	Tris-EDTA-Glucose (TEG) buffer	Refrigerator
F	Lysozyme	Freezer
G	Protein sample buffer	Freezer
H	Unknown (ready for electrophoresis)	Freezer

• Tris-glycine-SDS electrophoresis buffer (10x)	Room temperature
• Protein InstaStain®	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, 100 x 15 mm

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 recommended)
- Microcentrifuge
- Incubation oven (37° C)
- Microcentrifuge tubes
- Hot plate, Microwave oven or Bunsen burner
- White light box
- Glass staining trays
- Distilled water
- Glacial acetic acid
- 95 - 100% Methanol

Background Information

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.

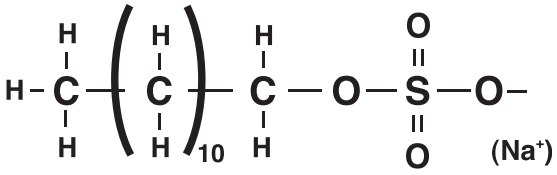


Figure 1 - The chemical structure of sodium dodecylsulfate (SDS).

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 concentrations of reducing agents, such as 2-mercaptoethanol, wll 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

Background Information

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.

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 subtilus is a gram positive, rod shaped member of the family Bacillaceae. Members of this family are endospore formers. *B.subtilus* 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.

Background Information

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.

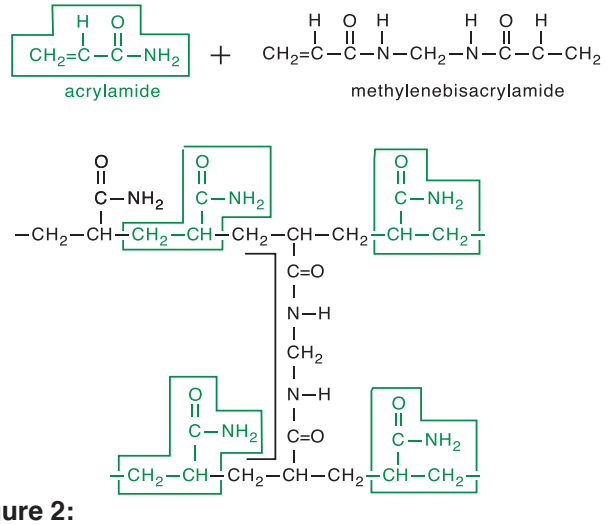


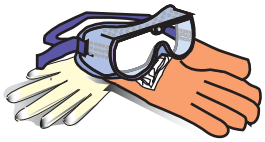
Figure 2:
Polymerization of polyacrylamide.

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.

LABORATORY SAFETY



Gloves and goggles should be worn routinely as good laboratory practice.

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 an unknown protein lysate will be compared in parallel with 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.

Experiment Procedure

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 slants 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 subtilus*

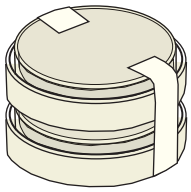
Preparation of Bacterial Lysates

5. Stack your group's of plates on top of one another and tape them together.

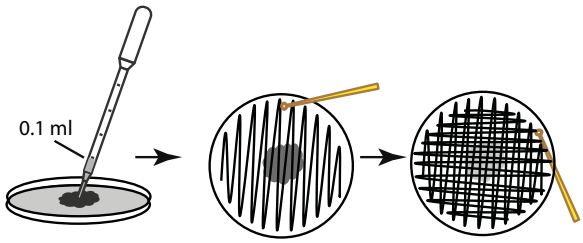
   The plates should be left in the upright position to allow the cell suspension to be absorbed by the agar.

6. Place the set of plates in a safe place designated by your instructor.

7. After the cell suspension is absorbed by the agar, you or your instructor will place the plates in the **inverted** position (agar side on top) in a 37° C bacterial incubation oven for overnight incubation (15-20 hours).

   If the cells have not been absorbed into the medium, it is best to incubate the plates upright. The plates are inverted to prevent condensation on the lid, which could drip onto the culture and may interfere with experimental results.



NOTE: Ensure the entire plate has been completely streaked over with the inoculating loop.

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.

1. 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).
2. Add 4 ml of tris-EDTA-glucose (TEG) to each plate.
3. Using a sterile loop for each plate, scrape the cells from the agar surface by streaking back and forth.
4. 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.
5. 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.
6. 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.
7. Place your microcentrifuge tubes in the microfuge, counter-balanced and centrifuge for 5 minute at full speed.
8. Remove all of the supernatant from the tubes with a pipet. Discard the supernants in a waste beaker.
9. Invert the tubes and blot residual liquid with a wipe or paper towel.
10. Determine the wet mass of the cell pellets.

NOTE: The wet mass of cell pellets may vary among the bacteria. It is recommended that the cell pellets be comparable in weight to ensure a consistent intensity of the protein bands in the final results. Suggested wet mass is between 0.020 and 0.025 grams.

Experiment Procedure

Preparation of Bacterial Lysates

11. 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.

12. Add one-tenth the volume of lysozyme solution to the cell resuspensions (e.g. 25 µl to 0.25 ml). Mix. Incubate in a 37° C water bath for 30 minutes.

13. Add three times the volume of protein sample buffer (contains SDS and 2 mercapto-ethanol) to the cell suspensions (e.g. 0.75 ml of buffer to 0.25 ml of cells, volume of lysozyme need not be considered).

14. Tighten the screw caps on the tubes. Suspend your sample tubes in a boiling water bath for 10 minutes.

15. Allow the tubes to cool. Centrifuge at full speed, 5 minutes in a microfuge.

16. Transfer most of the supernant to a fresh tube for storage. This is your protein lysate. 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.

Optional Dilution of Protein Lysates

The yield of the protein lysates can vary among student groups and greatly depends on how well the preparation of the bacterial lysates is performed.

Prior to the protein denaturing procedure (below), teachers may wish to compare the final results by assigning various student groups to set up different dilutions of the protein lysates using the Protein Sample Buffer (Component G).

Suggested dilution range is 25% to 50% dilution of the protein supernatant collected in step 21 (page 10). For example, if a 25% dilution is required for a protein lysate, 3 parts of protein supernatant should be added to the one part of the Protein Sample Buffer.

Protein Denaturation

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

- If the Unknown (component H) has already been rehydrated and heated by your lab instructor, proceed to denaturing the protein lysates as instructed in steps 1-2 below.
- If the Unknown (component H) has NOT been rehydrated and heated by your lab instructor, proceed to denaturing the Unknown, along with the protein lysates as instructed in steps 1-2 below.

1. Bring a beaker of water, covered with aluminum foil, to a boil. Remove from heat.
2. Suspend tubes containing the Unknown (Component H) along with the protein lysates (and/or their dilutions) in a boiling bath. Make sure the tubes 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.

NOTE: 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 re-heat and run the samples when using them at a later time.

Electrophoresis of Proteins

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.

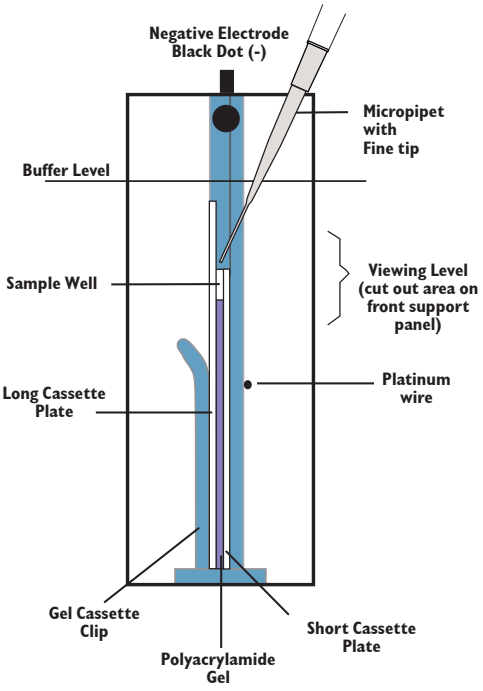
PROPER ORIENTATION OF THE GEL IN THE ELECTROPHORESIS UNIT

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.



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

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 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 µl of unknown (H)
Lane 2	15 µl of *E. coli* (A)
Lane 3	15 µl of *S.marcescens* (B)
Lane 4	15 µl of *M.luteus* (C)
Lane 5	15 µl of *B.subtilus* (D)

Group B

Lane 6	20 µl of unknown (H)
Lane 7	15 µl of *E. coli* (A)
Lane 8	15 µl of *S.marcescens* (B)
Lane 9	15 µl of *M.luteus* (C)
Lane 10	15 µl of *B.subtilus* (D)

QUICK REFERENCE:

Protein Samples:

A.	*E.coli*
B.	*S.marascens*
C.	*M.luteus*
D.	*B.subtilus*
H.	Unknown

RUNNING THE GEL

1. 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.

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

3. Set the power supply at the required voltage and run the electrophoresis for the length of time as determined by your instructor. When the current is flowing, you should see bubbles forming on the electrodes. The sudsing is due to the SDS in the buffer.

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

Time and Voltage

Volts	Recommended Time	
	Minimum	Optimal
125	60 min	75 min

Staining the Gel

Wear gloves
and safety goggles

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

Experiment Procedure

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. 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.)

Instructor's Guide

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 purchase precast gels (Cat. #s 651 or 652).

The experimental procedures consist of three major parts:
1) separation of proteins on polyacrylamide gels,
2) staining of protein bands,
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 staining method available exclusively from EDVOTEK®. You may wish to compare the staining methods by assigning various student groups to use different staining methods.

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.

PRACTICE GEL LOADING

This experiment kit contains practice gel loading solution. If your students are unfamiliar with vertical gel electrophoresis, it is suggested that they practice loading the sample wells before performing the actual experiment.

Pre-Lab Preparations

Caution!

WEAR GOGGLES AND HOT GLOVES!

PREPARATION OF AGAR PLATES
Within one week of lab

Student participation is suggested if possible. Students can prepare agar plates on the first day of the lab. However, plates should be allowed to solidify for an hour before streaking.

1. Loosen, but do not remove, the cap on the ReadyPour™ medium bottle. This will allow for the venting of steam during heating.

2. Place the bottle in a boiling (100° C) water bath or oven to completely melt the ReadyPour medium. Using a hot glove, occasionally VERY GENTLY swirl to expedite melting.

   Alternatively, you may microwave at medium setting to heat the bottle. Using a hot glove, occasionally VERY GENTLY swirl the bottle. **The ReadyPour medium will boil out of the bottle if left unattended.**

3. Allow the ReadyPour medium to cool to approximately 55° C (warm to the touch but not burning hot).

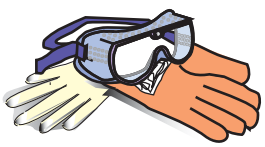
4. While the ReadyPour medium is cooling, label a total of 12 petri plates on their bottom halves:

   3 Plates for A - *Escherichia coli*
   3 Plates for B - *Serratia marcescens*
   3 Plates for C - *Micrococcus luteus*
   3 Plates for D - *Bacillus subtilus*

5. When the Ready Pour has cooled to approximately 55° C transfer approximately 12 ml of the medium to each of the 12 plates using a sterile 10 ml pipet. Rock the plate back and forth while adding the medium to obtain complete coverage.

6. Re-cover the plates and allow the medium to solidify.

7. Tape the plates and store them, inverted in the refrigerator. The plates should be used within 1 week.



Wear gloves and safety goggles

Especially during steps involving boiling or heating with microwave ovens or hot plates.

Pre-Lab Preparations

BACTERIAL SLANTS
First day, within 1 hour before the lab.

1. Using a different, sterile 1 ml pipet for each slant, add 1 ml of nutrient broth to slants A-D. Wait for 5 to 10 minutes before proceeding to step 2.

2. Use a sterile loop to enter slant A and thoroughly rub the entire immersed surface of the agar slant so the cells are released into the liquid. Close the vial and gently mix.

3. Using fresh loops, repeat step 2 for each of the slants B-D using a new sterile loop for each slant.

General comments for the first day of lab and group assignments.

Equilibrate a 37° C incubation oven. The slants will be passed between student groups.

Groups 1, 3 and 5:	(2) Plates and slant samples A, B
Groups 2, 4 and 6:	(2) Plates and slant samples C, D

If the students do not prepare the agar plates, day 1 of the lab will be relatively short (approximately 30 minutes to 1 hour). Plan accordingly.

RECONSTITUTION OF UNKNOWN SAMPLE (COMPONENT H)
Second day

1. Add 130 µl of distilled or deionized water to the tube containing the Unknown (H) and allow the sample 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. Make sure the sample tube is tightly capped and well labeled. The bottom of the tube should be pushed through the foil and immersed in the boiling water for 5 minutes. The tube should be kept suspended by the foil.

3. Aliquot 20 µl of sample per student group. Have students load the sample onto the polyacrylamide gel with it is still warm to avoid aggregation.

4. Store any unused portion of reconstituted sample at -20° C and repeat steps 2 and 3 when using samples at a later time.

RECONSTITUTION OF UNKNOWN SAMPLE (COMPONENT H)
Second day

Thaw the Protein Sample Buffer (G) at room temperature.

1. Using a permanent marker, label six (6) microcentrifuge tubes "buffer".
2. Aliquot 3 ml of Protein Sample Buffer (G) to each tube.
3. Distribute one "buffer" tube per student group.

PreLab Preparations

LYSOZYME
Second day, within 1 hour of lab.

1. Transfer 10 ml of cold TEG buffer (E) to a clean beaker, flask or test tube.
2. Add all the contents of tube F (lysozyme) to the buffer. Mix.
3. Store the enzyme solution on ice.
4. Aliquot 1.5 ml for each group.
5. Equilibrate a 37° C water bath. Have extra centrifuge tubes on hand for counter balances.

Notes Regarding Day Two Activities:

Sterility is not required for preparing the lysate. The volume of samples generated by the students will probably be much greater than the volumes needed for electrophoresis. These samples can be stored long term in the freezer. More gels can be purchased or prepared and several electrophoresis labs demonstrating protein lysates can be performed.

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

Pre-Lab Preparations

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 right.

Time and Voltage

Volts	Recommended Time	
	Minimum	Optimal
125	60 min	75 min

PREPARING STAINING AND DESTAINING SOLUTIONS

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

1. **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.
2. **Destaining Solution**
   - Use the stock solution of Methanol, Glacial Acetic acid and distilled water (in Step 1) to destain the gel(s).

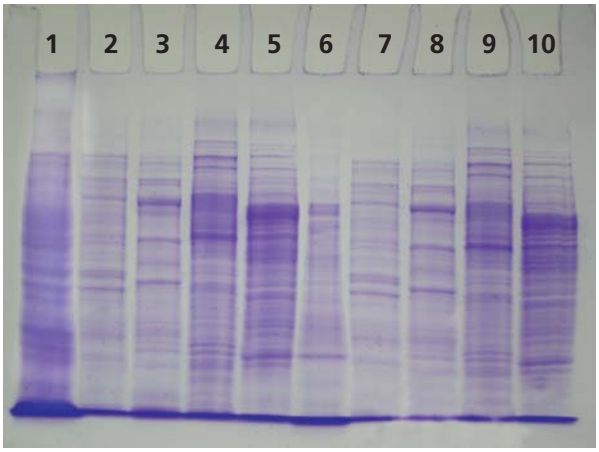
Idealized Schematic of Results

All protein lysates will contain many closely spaced bands. The molecular weight range of the lysate proteins extend from over 95,000 to below 14,000. Unknown (Component H) is a protein lysate from *E.coli.*

The gel below is an example of the different dilutions of the extracted protein lysates. A 25% dilution of the protein lysates is shown in lanes 2-5, and a 50% dilution of the protein lysates is shown in lanes 7-10.

Lanes	Sample
1	Unknown (Component H)
2	*E. coli*
3	*S.marcescens*
4	*M.luteus*
5	*B. subtilus*

Lanes	Sample
6	Unknown (Component H)
7	*E. coli*
8	*S.marcescens*
9	*M.luteus*
10	*B. subtilus*



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

EDVOTEK®

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

IDENTITY (As Used on Label and List): Protein InstaStain
Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.
Address (Number, Street, City, State, Zip Code): 1121 5th Street NW Washington DC 20001
Emergency Telephone Number: (202) 370-1500
Telephone Number for information: (202) 370-1500
Date Prepared: 10-24-11
Signature of Preparer (optional)

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Methanol (Methyl Alcohol) CH3OH	200ppm	200ppm	No data	90%-100%

Section III - Physical/Chemical Characteristics

Boiling Point	65°C	Specific Gravity (H_20 = 1)	.79
Vapor Pressure (mm Hg.)	96mmHg	Melting Point	N/A
Vapor Density (AIR = 1)	1.11	Evaporation Rate (Butyl Acetate = 1)	4.6

Solubility in Water: Complete (100%)
Appearance and Odor: chemical bound to paper, no odor

Section IV - Physical/Chemical Characteristics

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

Extinguishing Media: Use alcohol foam, dry chemical or carbon dioxide. (Water may be ineffective)
Special Fire Fighting Procedures: Wear SCBA with full facepiece operated in positive pressure mode. Move containers from firearea
Unusual Fire and Explosion Hazards: Vapors may flow along surfaces to distant ignition sources. Close containers exposed to heat may explode. Contact w/ strong oxidizers may cause fire.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	None

Incompatibility: Strong oxidizing agents
Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide, Sulfur oxides

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

Section VI - Health Hazard Data

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

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

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

Signs and Symptoms of Exposure: Respiratory tract: burning sensation. Coughing, wheezing, laryngitis, shortness of breath, headache
Medical Conditions Generally Aggravated by Exposure: No data
Emergency First Aid Procedures: Flush skin/eyes w/ large amounts of water. If inhaled, remove to fresh air. Ingestion: give large amounts of water or milk. Do not induce vomiting.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Evacuate area. Wear SCBA, rubber boots and rubber gloves. Mop up w/ absorptive material and burn in chemical incinerato equipped w/ an afterburner and scrubber.
Waste Disposal Method: Observe all federal, state, and local laws.
Precautions to be Taken in Handling and Storing: Wear protective gear. Avoid contact/inhalation.
Other Precautions: Strong sensitizer

Section VIII - Control Measures

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

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

Protective Gloves		Eye Protection	
	Rubber		Splash-proof goggles

Other Protective Clothing or Equipment: Rubber boots
Work/Hygienic Practices: Avoid prolonged or repeated exposure

EDVOTEK®

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

IDENTITY (As Used on Label and List): Practice Gel Loading Solution
Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.
Address (Number, Street, City, State, Zip Code): 1121 5th Street NW Washington DC 20001
Emergency Telephone Number: (202) 370-1500
Telephone Number for information: (202) 370-1500
Date Prepared: 10-24-11
Signature of Preparer (optional)

Section II - Hazardous Ingredients/Identify Information

This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_20 = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble
Appearance and Odor: Blue liquid, no odor

Section IV - Physical/Chemical Characteristics

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

Extinguishing Media: Dry chemical, carbon dioxide, water spray or foam
Special Fire Fighting Procedures: Use agents suitable for type of surrounding fire. Keep upwind, avoid breathing hazardous sulfur oxides and bromides. Wear SCBA.
Unusual Fire and Explosion Hazards: Unknown

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	None

Incompatibility: None
Hazardous Decomposition or Byproducts: Sulfur oxides, and bromides

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

Section VI - Health Hazard Data

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

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

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

Signs and Symptoms of Exposure: May cause skin or eye irritation
Medical Conditions Generally Aggravated by Exposure: None reported
Emergency First Aid Procedures: Treat symptomatically and supportively. Rinse contacted area with copious amounts of water.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear eye and skin protection and mop spill area. Rinse with water.
Waste Disposal Method: Observe all federal, state, and local regulations.
Precautions to be Taken in Handling and Storing: Avoid eye and skin contact.
Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

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

Protective Gloves		Eye Protection	
	Yes		Splash proof goggles

Other Protective Clothing or Equipment: None required
Work/Hygienic Practices: Avoid eye and skin contact

EDVOTEK®

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

IDENTITY (As Used on Label and List): Tris-Glycine SDS Running Buffer (10X)
Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.
Address (Number, Street, City, State, Zip Code): 1121 5th Street NW Washington DC 20001
Emergency Telephone Number: (202) 370-1500
Telephone Number for information: (202) 370-1500
Date Prepared: 10-24-11
Signature of Preparer (optional)

Section II - Hazardous Ingredients/Identify Information

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

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_20 = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble
Appearance and Odor: Clear, no odor

Section IV - Physical/Chemical Characteristics

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

Extinguishing Media: Water spray, carbon dioxide, dry chemical powder or appropriate foam
Special Fire Fighting Procedures: Wear SCBA and protective clothing
Unusual Fire and Explosion Hazards: May emit toxic fumes

Section V - Reactivity Data

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

Incompatibility: Strong oxidizing agents
Hazardous Decomposition or Byproducts: Carbon monoxide, carbon dioxide, sulfur oxides, sodium oxides

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	Will Not Occur	X	

Section VI - Health Hazard Data

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

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

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

Signs and Symptoms of Exposure: Irritation
Medical Conditions Generally Aggravated by Exposure: Unknown
Emergency First Aid Procedures: Skin/eye contact: flush w/ water. Inhalation: remove to fresh air. Ingestion: Seek medical attention

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear protective clothing. Avoid contact. Mop up with absorbant material and dispose of properly.
Waste Disposal Method: Follow all state, federal, and local regulations.
Precautions to be Taken in Handling and Storing: Avoid contact, keep away from heat.
Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

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

Protective Gloves		Eye Protection	
	Chem resistant		Safety goggles

Other Protective Clothing or Equipment: Lab coat, coveralls
Work/Hygienic Practices: Prevent contact

EDVOTEK®

Material Safety Data Sheet

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

IDENTITY (As Used on Label and List): 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.

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: 10-24-11

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Lysozyme ---------- No data ----------				
CAS # 12650-88-3				

Section III - Physical/Chemical Characteristics

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

Solubility in Water: Soluble

Appearance and Odor: Clear liquid, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	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

Section V - Reactivity Data

			Conditions to Avoid
Stability	Unstable		
	Stable	X	No data

Incompatibility: None

Hazardous Decomposition or Byproducts: No data

			Conditions to Avoid
Hazardous Polymerization	May Occur		
	Will Not Occur	X	No data

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): Prolonged or repeated exposure may cause allergic reactions in sensitive individuals

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

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released or 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

Section VIII - Control Measures

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