EDVOTEK®

Sci-On® Biology

S-45

EDVO-Kit #

What Size Are Your Genes?

Storage:
Store this experiment at room temperature

EXPERIMENT OBJECTIVE:

The objective of this experiment is to develop an understanding that genetic mutations are inherited from one or both parents. Mutations can include size rearrangements, which can be detected by gel electrophoresis.

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.

Table of Contents

Experiment Components

Storage:
Store entire experiment at room temperature.

ELECTROPHORESIS SAMPLES

- Ready-to-Load™ Dye samples representing genes

  A Standard dyes with assigned base pair equivalents
  B Gene 1
  C Gene 2
  D Gene 3
  E Gene 4
  F Gene 5

REAGENTS & SUPPLIES:

- Practice Gel Loading Solution
- UltraSpec-Agarose™ powder
- Concentrated electrophoresis buffer
- 1 ml pipet
- 100 ml graduated cylinder (packaging for samples)
- Microtipped Transfer Pipets

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA.

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips
- Balance
- Microwave, hot plate or burner
- Pipet pump
- 250 ml flasks or beakers
- Hot gloves
- DNA visualization system (white light)
- Distilled or deionized water

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.

Genes and Gene Mutations

Background Information

The genetic make up of an individual is inherited from both parents. Gene composition and genetic disease follows Mendelian inheritance patterns. An individual can inherit different combinations of genes: 1) two normal genes, 2) a normal gene and a modified (mutated) form, or 3) two mutated genes.

A person with one mutated gene often will not show clinical symptoms for the particular disease, but will be a carrier of the mutant gene. If an individual has two mutated genes, all the protein product will be in the mutated form. If the gene codes for a critical protein, the individual with two mutated genes often will suffer from a clinical disease. A copy of the mutated gene will also be inherited by the next generation.

Genetic diseases can be caused by a single base substitution, as in sickle cell anemia, or a rearranged gene that has deleted (truncated) sequences, such as in certain cancers. Environmental factors, such as carcinogens or certain viruses, can contribute to non-inherited gene modifications.

The identification of specific mutations or gene rearrangements of certain genes are being developed as medical diagnostic tests to detect predisposition of disease states. Size determination of DNA fragments and genes are also essential for genetic engineering and DNA technology-based experiments. Additional examples of the applications of this technology include DNA fingerprinting, human diagnostics and tests for the predisposition of genetically inherited diseases.

Agarose gel electrophoresis can rapidly and easily detect differences in the size of genes. The gel is made by dissolving agarose powder in boiling buffer solution. The solution is then cooled to approximately 55°C and poured into a mold where it solidifies. The gel is submerged in a buffer-filled chamber which contains electrodes.

The samples are loaded with a micropipet or transfer pipet into wells created in the gel by a template during casting. Samples are prepared for electrophoresis by mixing them with components that give the mixture density, such as glycerol or sucrose. This makes the samples denser than the electrophoresis buffer, so they sink through the buffer and remain in the wells.

A direct current power supply is connected to the electrophoresis apparatus and current is applied. Charged DNA or dye samples

Background Information

enter the gel through the walls of the wells. Molecules having a net negative charge, such as DNA or negatively charged dyes, migrate towards the positive electrode (anode) while net positively charged molecules migrate towards the negative electrode (cathode). Within a range, the higher the applied voltage, the faster the samples migrate. The buffer serves as a conductor of electricity and to control the pH. The pH is important to the charge and stability of biological molecules.

Agarose is a polysaccharide derivative of agar. In this experiment, UltraSpec Agarose™ is used. This material is a mixture of agarose and hydrocolloids which renders the gel to be both clear and resilient. The gel contains microscopic pores which act as a molecular sieve. The sieving properties of the gel influences the rate at which a molecule migrates. Smaller molecules move through the pores more easily than larger ones. This means that the smaller the molecule, the faster it migrates through the gel. Molecules can have the same molecular weight and charge but different shapes. Molecules having a more compact shape (a sphere is more compact than a rod) can move more easily through the pores.

Quick Reference:

The Standard dyes have the following base pair equivalents.

Blue 1	3,500
Red	1,500
Purple 1	800
Yellow 1	450

In this experiment, dyes representing normal and mutated genes are separated by electrophoresis. The dyes are negatively charged and will migrate through the gel in the same directly (towards the positive electrode) as DNA fragments. They will be separated according to their respective size and net charge. The sizes of the two copies of a gene (dyes) can be estimated visually based on their relative migration when compared to standard dyes of known sizes.

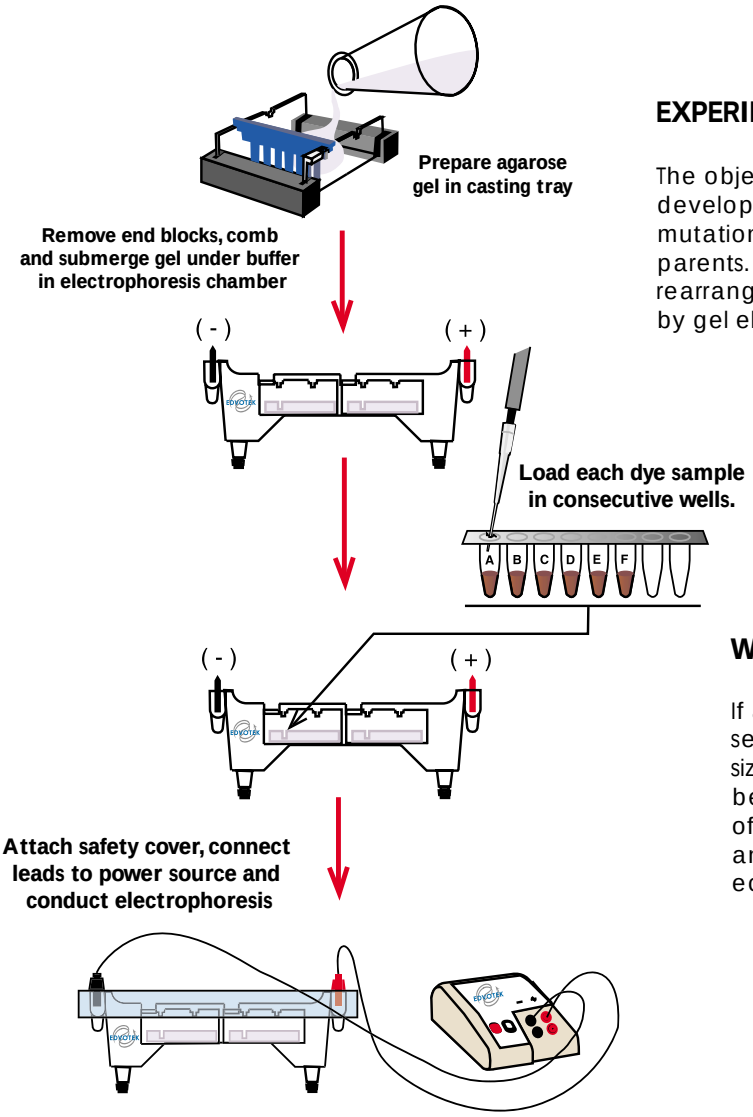
In an additional activity, migration distances of the dyes representing genes can be measured and plotted on semi-log graph paper. Dyes with assigned sizes (standard dyes) are used to plot a standard curve. The sizes of the various dyes ("genes") can be obtained by measuring their mobility and determining the point of intersection on the standard curve.

Experiment Overview

Experiment Procedures

BEFORE YOU START THE EXPERIMENT

1. Read all instructions before starting the experiment.
2. Write a hypothesis that reflects the experiment and predict experimental outcomes.



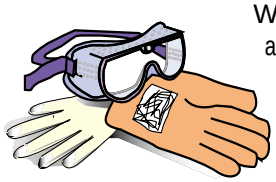
EXPERIMENT CONTENT OBJECTIVE

The objective of this experiment is to develop an understanding that genetic mutations are inherited from one or both parents. Mutations can include size rearrangements, which can be detected by gel electrophoresis.

WORKING HYPOTHESIS

If agarose gel electrophoresis separates molecules according to size, then semilog graph paper can be used to plot migration distances of known and unknown molecules and the estimated sizes of molecules determined.

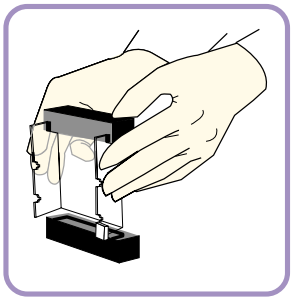
Activity One - Agarose Gel Preparation and Practice Gel Loading


Wear gloves and safety goggles

LABORATORY SAFETY

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

PREPARING THE GEL BED



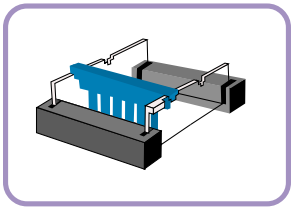
1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.

   A. Using Rubber dams:

   - Place a rubber dam on each end of the bed. Make sure the rubber dam fits firmly in contact with the sides and bottom of the bed.

   B. Taping with labeling or masking tape:

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



2. Place a well-former template (comb) in the first set of notches at the end of the bed. Make sure the comb sits firmly and evenly across the bed.

Experiment Procedures

Activity One - Agarose Gel Preparation and Practice Gel Loading

Experiment Procedures

CASTING AGAROSE GELS

3. Use a 250 ml flask to prepare the gel solution. Add the following components to the flask as specified for your experiment (refer to Table A).
    - Buffer concentrate
    - Distilled water
    - Agarose powder

Table A Individual 0.8% UltraSpec-Agarose™ Gel Electrophoresis of Dyes

Size of EDVOTEK Casting Tray (cm)	Amt of Agarose (g)	+	Concentrated Buffer (50x) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
7 x 7	0.24		0.6		29.4		30
7 x 15	0.48		1.2		58.8		60

4. Swirl the mixture to disperse clumps of agarose powder.

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

6. Heat the mixture to dissolve the agarose powder. The final solution should appear clear (like water) without any undissolved particles.

    A. Microwave method:
    - Cover the flask with plastic wrap to minimize evaporation.
    - Heat the mixture on High for 1 minute.
    - Swirl the mixture and heat on High in bursts of 25 seconds until all the agarose is completely dissolved.

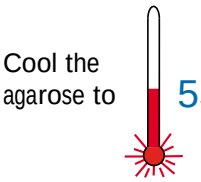
    B. Hot plate method:
    - Cover the flask with aluminum foil to prevent excess evaporation.
    - Heat the mixture to boiling over a burner with occasional swirling. Boil until all the agarose is completely dissolved.

    Check the solution carefully. If you see "crystal" particles, the agarose is not completely dissolved.

At high altitudes, it is recommended to use a microwave oven to reach boiling temperatures.

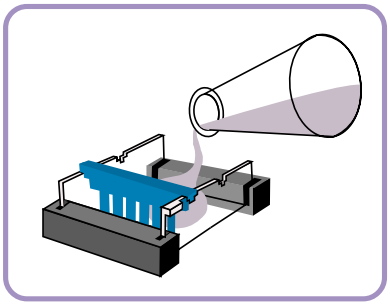
Activity One - Agarose Gel Preparation and Practice Gel Loading

7. Cool the agarose solution to 55°C with careful swirling to promote even dissipation of heat. If detectable evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 5.



DO NOT POUR BOILING HOT AGAROSE INTO THE GEL BED.

Hot agarose solution may irreversibly warp the bed.



After the gel is cooled to 55°C:

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

8. Seal the interface of the gel bed and tape to prevent the agarose solution from leaking.
   - Use a transfer pipet to deposit a small amount of cooled agarose to both inside ends of the bed.
   - Wait approximately 1 minute for the agarose to solidify.
9. Pour the cooled agarose solution into the bed. Make sure the bed is on a level surface.
10. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes.

Experiment Procedures

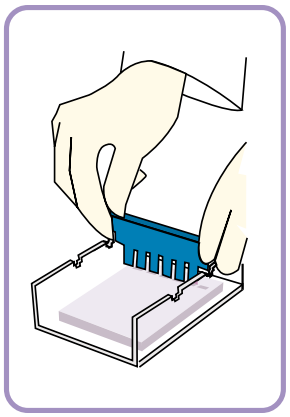
Activity One - Agarose Gel Preparation and Practice Gel Loading

Experiment Procedures

PREPARING THE GEL FOR ELECTROPHORESIS

11. After the gel is completely solidified, carefully and slowly remove the rubber dams or tape from the gel bed.

    Be especially careful not to damage or tear the gel wells when removing the rubber dams. A thin plastic knife, spatula or pipet tip can be inserted between the gel and the dams to break possible surface tension.



12. Remove the comb by slowly pulling straight up. Do this carefully and evenly to prevent tearing the sample wells.

13. Place the gel (on its bed) into the electrophoresis chamber, properly oriented, centered and level on the platform.

14. Fill the electrophoresis apparatus chamber with the required volume of diluted buffer for the specific unit you are using (see guidelines in Table B).

    For DNA analysis, the same EDVOTEK 50x Electrophoresis Buffer is used for preparing both the agarose gel buffer and the chamber buffer. The formula for diluting EDVOTEK (50x) concentrated buffer is 1 volume of buffer concentrate to every 49 volumes of distilled or deionized water.

The electrophoresis (chamber) buffer recommended is Tris-acetate-EDTA (20 mM tris, 6 mM sodium acetate, 1 mM disodium ethylenediamine tetraacetic acid) pH 7.8. Prepare the buffer as required for your electrophoresis apparatus.

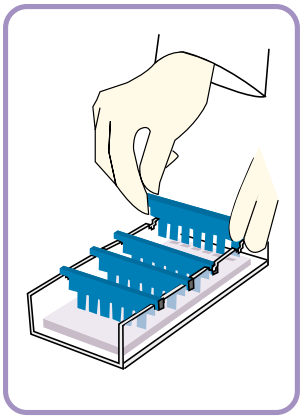
Table B Dilution of Electrophoresis (Chamber) Buffer

EDVOTEK Model #	Concentrated Buffer (50x) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
M6+	6		294		300
M12	8		392		400
M36 (blue)	10		490		500
M36 (clear)	20		980		1000

15. Make sure the gel is completely covered with buffer.

16. Proceed to loading the samples and conducting electrophoresis.

Activity One - Agarose Gel Preparation and Practice Gel Loading



PRACTICE GEL LOADING

Accurate sample delivery technique ensures the best possible gel results. Pipeting mistakes can cause the sample to become diluted with buffer, or cause damage to the wells with the pipet tip while loading the gel.

If you are unfamiliar with loading samples in agarose gels, it is recommended that you practice sample delivery techniques before conducting the actual experiment. EDVOTEK electrophoresis experiments contain a tube of practice gel loading solution for this purpose. Casting of a separate practice gel is highly recommended. One suggested activity is outlined below:

1. Cast a gel with the maximum number of wells possible.
2. After the gel solidifies, place it under buffer in an electrophoresis apparatus chamber.

   Alternatively, your teacher may have cut the gel in sections between the rows of wells. Place a gel section with wells into a small, shallow tray and submerge it under buffer or water.

   Note: The agarose gel is sometimes called a "submarine gel" because it is submerged under buffer for sample loading and electrophoretic separation.
3. Practice delivering the practice gel loading solution to the sample wells. Take care not to damage or puncture the wells with the pipet tip.

   - For electrophoresis of dyes, load the sample well with 35-38 microliters of sample.
   - If using transfer pipets for sample delivery, load each sample well until it is full.
4. If you need more practice, remove the practice gel loading solution by squirting buffer into the wells with a transfer pipet.
5. Replace the practice gel with a fresh gel for the actual experiment.

   Note: If practice gel loading is performed in the electrophoresis chamber, the practice gel loading solution will become diluted in the buffer in the apparatus. A small amount of practice gel loading solution (filling up to 12 wells) will not interfere with the experiment, so it is not necessary to prepare fresh buffer.

See the following page for specific instructions regarding the operation of an automatic micropipet.

If you are using transfer pipets, gently squeeze the pipet stem, instead of the bulb to help control the delivery of small sample volumes.

Activity Two - Conducting Agarose Gel Electrophoresis

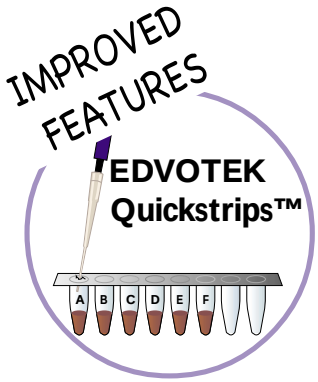
Experiment Procedures

ELECTROPHORESIS SAMPLES

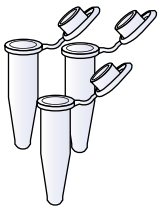
Samples in EDVOTEK Series 100 and S-series electrophoresis experiments are packaged in one of two different formats:

1. Pre-aliquoted Quickstrip™ connected tubes (new format)

   To remove samples from the Quickstrip™ tubes, simply pierce the foil top with the micropipet tip and withdraw the sample.

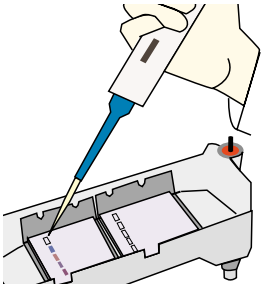


Quickstrips patent pending



2. Individual 1.5 ml or 0.5 ml microtest tubes

   Your instructor may have aliquoted these into a set of sample tubes for each lab group. Alternatively, you may be required to withdraw the appropriate amount from the experiment stock tubes.



LOADING THE SAMPLES

1. Check the Sample Volumes

   Sometimes a small amount of sample will cling to the walls of the tubes. Make sure the entire volume of sample is at the the bottom of the tubes before starting to load the gel.

   - If your samples are in Quickstrip™ connected tubes, tap the foil top of the strip so samples fall to the bottom of the tubes.
   - If your samples are in individual 1.5 ml or 0.5 ml microtest tubes, briefly centrifuge the sample tubes, or tap each tube on the tabletop to get all the sample to the bottom of the tube.

2. Load Samples

   Load each of the dye samples in tubes A - F into the wells in consecutive order. The amount of sample that should be loaded is 35-38 µl.

Lane	Label	Sample
1	A	Standard Marker Dyes
2	B	Gene 1
3	C	Gene 2
4	D	Gene 3
5	E	Gene 4
6	F	Gene 5

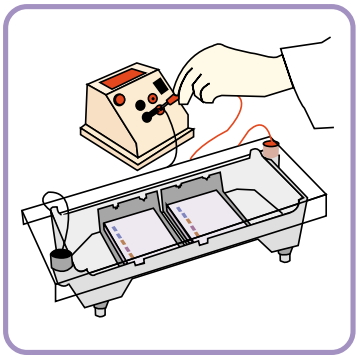
Activity Two - Conducting Agarose Gel Electrophoresis

RUNNING THE GEL

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

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

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



5. Set the power source at the required voltage and conduct electrophoresis for the length of time determined by your instructor. General guidelines are presented in Table C.

6. Check to see that current is flowing properly - you should see bubbles forming on the two platinum electrodes.

Table C Time and Voltage

Electrophoresis of Dyes

Volts	Recommended Time
125	20 min
70	45 min
50	1 hr 30 min

7. After approximately 10 minutes, you will begin to see separation of the colored dyes.

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

9. Document the gel results.

   A variety of documentation methods can be used, including drawing a picture of the gel, taking a photograph, or scanning an image of the gel on a flatbed scanner.

Staining is not required for Experiment # S-45, but results must be analyzed upon completion of the electrophoretic separation. Because dye molecules are extremely small they will diffuse out of the gel. Therefore, the gel cannot be saved.

Activity Three - Size Determination of Dyes ("Genes")

Experiment Procedures

This exercise focuses on the first steps for mapping, which is used in the Human Genome Project. The assignment of sizes for the dyes separated by agarose gel electrophoresis can have ± 10% margin of error. The sizes of the "unknowns" are extrapolated by their migration distances relative to the Standard dyes (Sample A), for which the size of each fragment is assigned and known.

Quick Reference:

The Standard dyes have the following base pair equivalents.

Blue 1	3,500
Red	1,500
Purple 1	800
Yellow 1	450

1. Measure and record the distance traveled in the agarose gel by each Standard dye.

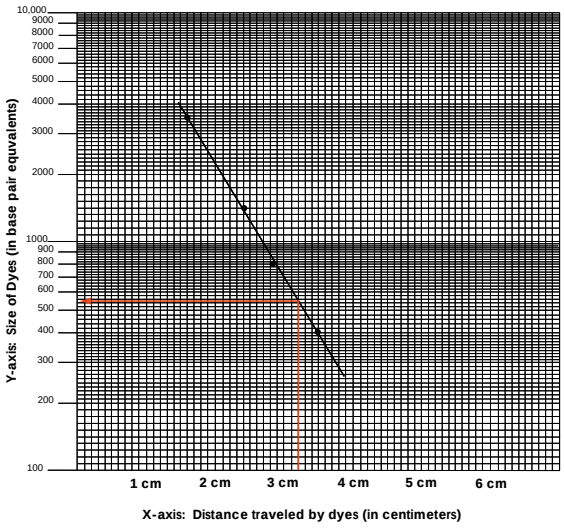
   In each case, measure from the lower edge of the sample well to the lower end of each band. Record the distance traveled in centimeters (to the nearest millimeter).

2. Use semi-log paper to graph your gel results. The non-logarithmic horizontal x-axis is labeled "Distance travelled by dyes" in centimeters at equal intervals. The logarithmic vertical y-axis is labeled "Size of Dyes".

3. For each Standard dye, plot the measured migration distance on the x-axis versus its size in base pairs, on the y-axis.

4. Draw the best average straight line through all the points.

   The line should have approximately equal numbers of points scattered on each side of the line. Some points may be right on the line (see the example at left).



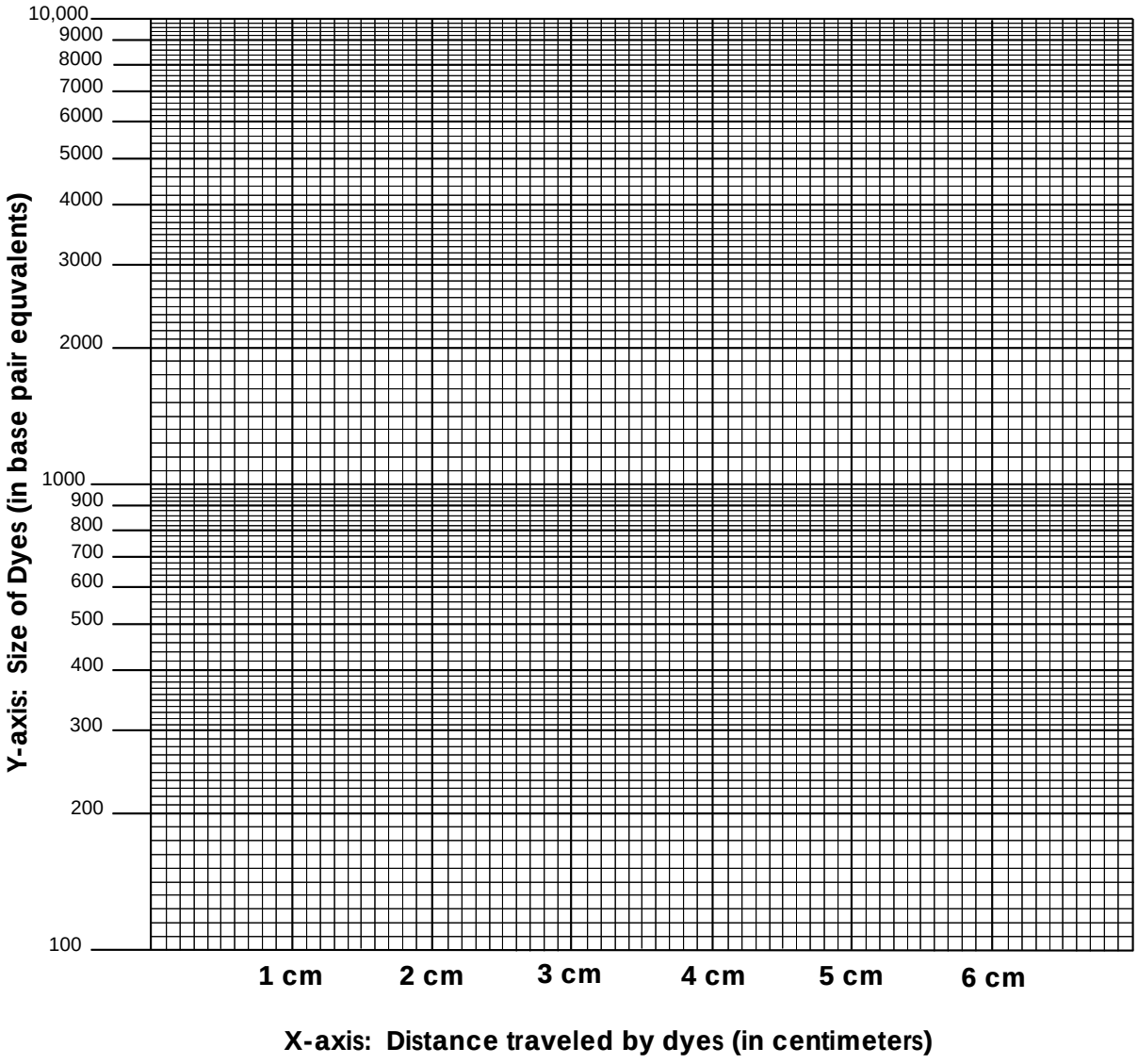
5. Measure the migration distance of each of the "unknown" dyes from samples B, C, D, E and F.

6. Using the graph of the Standard dyes below, plot and determine the sizes of each "unknown" dye.

   A. Find the migration distance of the unknown dye on the x-axis, and draw a vertical line from that point until the standard graph line is intersected.

   B. From the point of intersection, draw a second line horizontally to the y-axis and determine the approximate size of the dye in base pair equivalents.

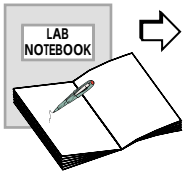
Activity Three - Size Determination of Dyes (“Genes”)



Experiment Procedures

Experiment Procedures

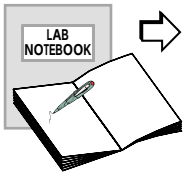
Critical Thinking and Hypothesis Development



Record the following in your Laboratory Notebook* or as instructed by your teacher.

1. What is the variable in this experiment?
2. What is the control in this experiment?
3. What could one change in the experiment if this experiment was repeated?
4. Write a hypothesis that would reflect a change.

Study Questions



Record the answers to the following Study Questions in your Laboratory Notebook* or as instructed by your teacher.

1. What is the function of the buffer in electrophoresis?
2. Why are the dyes moving in one direction, towards the positive electrode?
3. What impact would the concentration of agarose have on the mobility of molecules?
4. Why is the yellow dye in Sample C moving slower than the blue dye in Sample D?
5. When plotting the sizes of known and unknown fragments, which axis is used to plot the migration distances?
6. Which axis is used to plot the sizes of the known and unknown fragments?
7. How does the size of the molecule affect the migration rate of molecules?
8. Why is it useful to know sizes of genes?

*EDVOTEK Cat. # 1401 Laboratory DataBook is recommended.

Notes to the Instructor

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines include Suggestions for Lesson Plan Content which can be adapted to fit your specific set of circumstances.

APPROXIMATE TIME REQUIREMENTS

1. UltraSpec-Agarose™ gel preparation: Your schedule will determine when to prepare the gel(s) for an experiment. Whether you choose to prepare the gel(s) or have the students do it, allow approximately 30-40 minutes for this procedure. Generally, 20 minutes of this time is required for gel solidification.

2. The approximate time for electrophoresis will vary from 20 minutes to 1.5 hours.

ELECTROPHORESIS HINTS AND HELP

EDVOTEK Ready-to-Load Electrophoresis Experiments are easy to perform and are designed for maximum success in the classroom setting. However, even the most experienced students and teachers occasionally encounter experimental problems or difficulties.

The EDVOTEK web site provides a variety of resources which are continuously being updated and added. Several suggestions and reminders for conducting electrophoresis are available, as well as answers to frequently asked electrophoresis questions.

If you do not find the answers to your questions in this section or at the EDVOTEK web site, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

Technical Service Department

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

FAX: (301) 340-0582
web: www.edvotek.com
email: edvotek@aol.com

Please have the following information:

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

Suggestions for Lesson Plan Content

Instructor's Guide

This teacher-generated lesson plan outline can be used as a guideline for classroom discussion. Connections to the National Content and Skills Standards follow the plan.

1. Conduct a discussion of DNA, the material which controls who we are and what we look like.

2. Give students an overview of the structure of DNA.

3. Write the following words on the board for discussion:

Nucleus	Phosphodiester backbone
Chromosome	Base pair
Gene	Adenine
DNA	Guanine
Double helix	Cytosine
Nucleotide	Thymine
Deoxyribose	

   Use pictures or drawings from textbooks and other resources to help explain the vocabulary words to the students.

4. Focus on the following concepts to ensure student understanding, to maximize the benefits of the "hands-on" experience.

   - DNA is found in the cell nucleus in human cells and the nuclear area of bacteria.
   - DNA is made up of two strands of nucleotides that are held by ribose-phosphodiester backbones.
   - Each strand has a sequence of nucleotides (bases) placed in a specific order.
   - The bases are called Adenine (A), Guanine (G), Thymine (T), and Cytosine (C).
   - The two strands are joined together because the base pairs form physical bonds (known as hydrogen bonds) with each other in very specific ways. Adenine base-pairs with Thymine and Cytosine base-pairs with Guanine.

continued

Suggestions for Lesson Plan Content

- Once the strands are joined (base-paired), they twist to form a double helix because of physical forces on the DNA molecule.
- A chromosome contains many coding segments called genes.
- Each gene codes for a specific genetic trait.

5. List and discuss with students the essential parts of an experiment.

- writing a logical hypothesis
- making careful observations
- differentiating between an experiment and a control
- identifying variable(s)
- predicting experimental outcomes
- recording results in a concise and accurate manner
- drawing valid interpretations of results
- formulating alternative explanations

Instructor's Guide

Connections to National Content Standards

1. Students will develop abilities necessary to do scientific inquiry.
   - Student questions will be answered by conducting a scientific investigation.

2. Students will develop an understanding through inquiry.
   - Students will develop a logical hypothesis.
   - Students will make careful observations.
   - Students will interpret results correctly.
   - Students will understand the difference between the experiment and the control
   - Students will identify and control the variable.
   - Students will predict experimental outcomes.
   - Students will formulate explanations from results.
   - Students will recognize and analyze alternative explanations.

3. Students will use equipment, materials, and techniques for experimentation and direct investigation of phenomena.
   - Students will understand the principles of agarose electrophoresis.

4. Students will develop an understanding of the principles behind determination of fragment length sizes of molecules using agarose gel electrophoresis.
   - Students will use semi-log graph paper to plot results and determine the sizes of molecules using standard dye molecules

Connections to National Skill Standards

Students will learn to load and run agarose gel electrophoresis. Analysis of the experiment will provide students the means to transform an abstract concept into a concrete explanation.

Students will be able to:

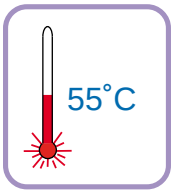
1. Use scientific equipment such as calibrated pipets for metric measurements and run electrophoresis.
2. Accurately load and run an agarose gel.
3. Make careful observations and record results.
4. Accurately measure migration distances of known and unknown fragments in a gel.
5. Accurately plot data on semi-log paper and determine fragment sizes.

Pre-Lab Preparations

BATCH AGAROSE GEL PREPARATION

To save time, the agarose gel solution can be prepared in a batch for sharing by the class. Any unused prepared agarose can be saved and remelted for gel casting at a later time. For a batch (375 ml) preparation of 0.8% agarose gel:

1. Use a 500 ml flask to prepare the diluted gel buffer.
   - Add 7.5 ml of buffer concentrate
   - Add 367.5 ml of distilled water.
2. Pour 3.0 grams of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as previously described for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 55°C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume (375 ml) as marked on the flask in step 3.



Note: The UltraSpec-Agarose™ kit component is often labeled with the amount it contains. In many cases, the entire contents of the bottle is 3.0 grams. Please read the label carefully. If the amount of agarose is not specified or if the bottle's plastic seal has been broken, weigh the agarose to ensure you are using the correct amount.

Table D Batch Preparation of 0.8% UltraSpec-Agarose™

Amt of Agarose (g)	+	Concentrated Buffer (50x) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
3.0		7.5		367.5		375

6. Dispense the required volume of cooled agarose solution for casting the gels. The volume required is dependent upon the size of the gel bed (refer to Table A for individual gel casting guidelines).
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Then proceed with preparing the gel for electrophoresis.

Instructor's Guide

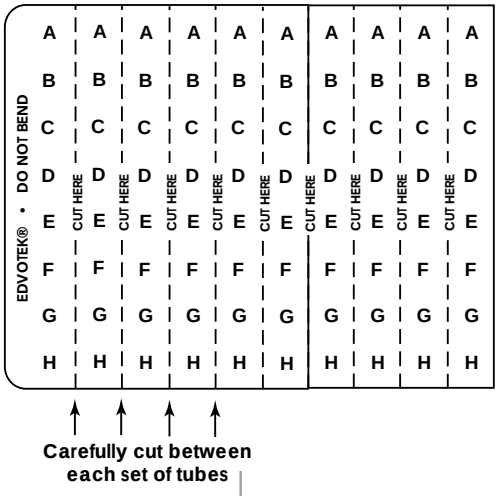
Instructor's Guide

Pre-Lab Preparations

Electrophoresis samples and reagents in EDVOTEK experiments are packaged in various formats. Samples in Series 100 and Sci-On electrophoresis experiments will be packaged in one of the following ways:

1) Pre-aliquoted Quickstrip™ connected tubes (new format)
OR
2) Individual 1.5 ml or 0.5 ml microtest tubes

IMPROVED FEATURES



FORMAT: PRE-ALIQUOTED QUICKSTRIP™ CONNECTED TUBES

If the Quickstrip™ samples are not already cut into individual strips:

1. Use sharp scissors to separate each set of tubes A-H in the block of samples.

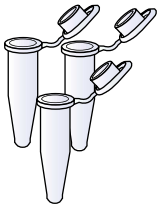
   Note: In this experiment, tubes G and H are empty.

2. Cut carefully through the foil between the rows of samples. Do not cut or puncture the foil covering the top of the sample tubes.

3. Each group will require one strip of samples.

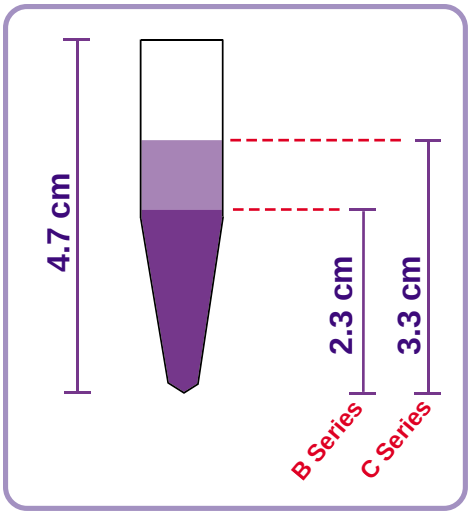
4. Remind students to tap the foil or tubes before gel loading to ensure that all of the sample is at the bottom of the tube.

Pre-Lab Preparations



FORMAT: INDIVIDUAL 1.5 ML MICROTEST TUBES

It is recommended that samples packaged in 1.5 ml individual microtest tubes be aliquoted for each gel. Samples packaged in this format include bulk samples for EDVOTEK Series 100 electrophoresis experiments and are available in two standard quantities: the B-Series (480 µl) and the C Series (960 µl). Custom bulk quantities are also available by request.



Before aliquoting, check all sample volumes for possible evaporation. The samples will become more concentrated if evaporation has occurred.

If needed, tap or centrifuge the sample tubes. Then add distilled water to slightly above the following level:

2.3 cm level for the B-Series

3.3 cm level for the C-Series

Mix well by inverting and tapping the tubes several times.

After checking sample volumes and determining that the samples are at their proper total volumes:

1. Aliquot the dye samples into appropriately labeled 0.5 ml or 1.5 ml microtest tubes:

   38-40 µl of each sample

2. Students might have difficulty retrieving the entire aliquoted volume of sample because some of it may cling to the side walls of the tubes. Some suggestions are:

   - Remind students to make sure all of the sample is at the bottom of the tube before gel loading. They should centrifuge the samples tubes, or tap the tubes on the tabletop.
   - Instruct students to set their automatic micropipets to a volume that is 2 microliters less than the volume you have aliquoted.

Pre-Lab Preparations

Instructor's Guide

MATERIALS FOR THE EXPERIMENT

Each Lab Group should have the following materials:

Activity One

- Agarose gel
- Electrophoresis Buffer
- Practice gel loading sample
- Sample delivery instrument
  - Automatic micropipet and tips, or
  - Transfer pipet and beaker of distilled water

Activity Two

- Agarose gel
- Electrophoresis apparatus
- DC power source
- Dye Samples (A - F) representing various DNA samples
- Sample delivery instrument
  - Automatic micropipet and tips, or
  - Transfer pipet and beaker of distilled water

Activity Three

- Drawing or photo of gel results
- Semilog graph
- Metric ruler

Notes regarding electrophoresis

1. Do not move the apparatus immediately after the samples have been loaded.
   - Moving the apparatus will dislodge the samples from the wells into the buffer and will compromise results.
   - If it is necessary to move the apparatus during electrophoresis, you may safely do so after the tracking dye has migrated at least 1 cm from the wells into the gel.

2. For optimal separation, do not use voltages higher than 125 volts for agarose gel electrophoresis. Higher voltages can overheat and melt the gel.

3. Electrophoresis should be terminated when the dyes have moved 3 to 4 centimeters from the wells and before it moves off the gel.

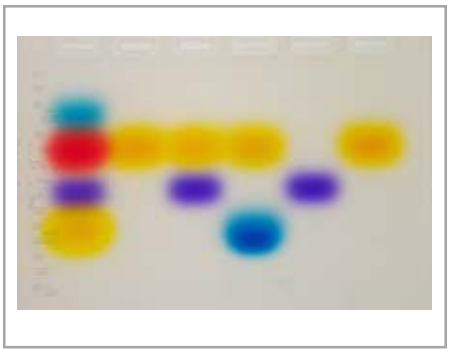
AVOIDING COMMON PITFALLS

Potential pitfalls and/or problems can be avoided by following the suggestions and reminders listed below.

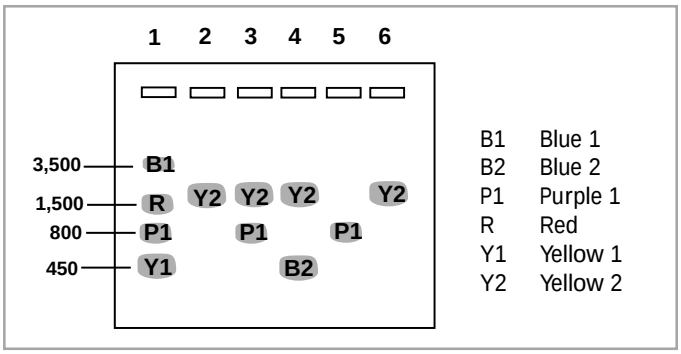
- To ensure that dyes are well resolved, make sure the gel formulation is correct (see Table A) and that electrophoresis is conducted for the optimal recommended amount of time.
- Correctly dilute the concentrated buffer for preparation of both the gel and electrophoresis (chamber) buffer. Remember that without buffer in the gel, there will be no sample mobility. Use only distilled water to prepare buffers. Do not use tap water.
- For optimal results, use fresh electrophoresis buffer prepared according to instructions.
- Before performing the actual experiment, practice sample delivery techniques to avoid diluting the sample with buffer during gel loading.
- To avoid loss of samples into the buffer, make sure the gel is properly oriented in the electrophoresis unit so the samples are not electrophoresed in the wrong direction off the gel.

Instructor's Guide

Experiment Results



S-45 gel result photo



S-45 Idealized schematic

Each lane represents an individual's make up for a particular gene. Each protein is coded by two genes that are inherited from both parents. If one of the genes is mutated, the person can still generate the correct protein (from the other non-mutant gene) and will not show a full blown clinical condition. The mutant gene can be inherited in a Mendellian pattern; if both genes have the same critical mutation, the individual will be a carrier of disease. In this experiment analysis is based on the size of the gene.

Note: This technique has a ± 10 - 15% margin of error.

Lane	Description
1	A set of standard dye makers of known sizes
2	Two copies of a normal gene (Yellow 2) obtained from both parents (one each)
3	One normal gene (Yellow 2) copy and a second (Purple 1) truncated form of the gene
4	One normal gene (Yellow 2) and second version truncated form of the gene (Blue 2)
5	Two copies of the truncated form of the gene (Purple 1). Person has the clinical symptoms
6	Two copies of the normal gene (Yellow 2)

Lane	Tube		Size	
1	A	Standard Marker Dyes*	3,500 1,500 800 450	
2	B	Gene 1	1850	± 278
3	C	Gene 2	1850 800	± 278 ± 120
4	D	Gene 3	1850 450	± 278 ± 68
5	E	Gene 4	800	± 120
6	F	Gene 5	1850	± 278

*expressed in assigned base pair equivalents

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): Agarose

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

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

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

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

Date Prepared: 07/01/03

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)

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

CAS #9012-36-6

Section III - Physical/Chemical Characteristics

Boiling Point: For 1% solution	194° F	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: Insoluble - cold

Appearance and Odor: White powder, no odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

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

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

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

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable	X	None

Incompatibility: No data available

Hazardous Decomposition or Byproducts:

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): Inhalation: No data available Ingestion: Large amounts may cause diarrhea

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: No data available

Medical Conditions Generally Aggravated by Exposure: No data available

Emergency First Aid Procedures: Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

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

Waste Disposal Method: Normal solid waste disposal

Precautions to be Taken in Handling and Storing: None

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator with full facepiece.

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

Protective Gloves: Yes Eye Protection: Splash proof goggles

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

Work/Hygienic Practices: None

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): **50x Electrophoresis Buffer**

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

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

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

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

Date Prepared: 07/01/03

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)

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

Section III - Physical/Chemical Characteristics

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

Solubility in Water: Appreciable, (greater than 10%)

Appearance and Odor: Clear, liquid, slight vinegar odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

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

Extinguishing Media: Use extinguishing media appropriate for surrounding fire.

Special Fire Fighting Procedures: Wear protective equipment and SCBA with full facepiece operated in positive pressure mode.

Unusual Fire and Explosion Hazards: None identified

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable	X	None

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): None

Carcinogenicity: None identified NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: Irritation to upper respiratory tract, skin, eyes

Medical Conditions Generally Aggravated by Exposure: None

Emergency First Aid Procedures: Ingestion: If conscious, give large amounts of water

Eyes: Flush with water Inhalation: Move to fresh air Skin: Wash with soap and water

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear suitable protective clothing. Mop up spill and rinse with water, or collect in absorptive material and dispose of the absorptive material.

Waste Disposal Method: Dispose in accordance with all applicable federal, state, and local enviromental regulations.

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

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

Protective Gloves: Yes Eye Protection: Safety goggles

Other Protective Clothing or Equipment: None

Work/Hygienic Practices: None

EDVOTEK®

Material Safety Data Sheet

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

IDENTITY (As Used on Label and List): **Practice Gel Loading Solution**

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

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

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

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

Date Prepared: 07/01/03

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)] | OSHA PEL | ACGIH TLV | Other Limits Recommended | % (Optional)

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

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_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: Blue liquid, no odor

Section IV - Physical/Chemical Characteristics

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

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid: None
	Stable	X	

Incompatibility: None

Hazardous Decomposition or Byproducts: Sulfur oxides, and bromides

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

Section VI - Health Hazard Data

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

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

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

Signs and Symptoms of Exposure: May cause skin or eye irritation

Medical Conditions Generally Aggravated by Exposure: None reported

Emergency First Aid Procedures: Treat symptomatically and supportively. Rinse contacted area with copious amounts of water.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear eye and skin protection and mop spill area. Rinse with water.

Waste Disposal Method: Observe all federal, state, and local regulations.

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type)

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

Protective Gloves: Yes | Eye Protection: Splash proof goggles

Other Protective Clothing or Equipment: None required

Work/Hygienic Practices: Avoid eye and skin contact

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): Orange G

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

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

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

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

Date Prepared: **07/01/03**

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)] | OSHA PEL | ACGIH TLV | Other Limits Recommended | % (Optional)

This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard. CAS # 1936-15-8

Section III - Physical/Chemical Characteristics

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

Solubility in Water: Soluble

Appearance and Odor: yellow-orange color, liquid, no odor

Section IV - Physical/Chemical Characteristics

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

Extinguishing Media: N/A

Special Fire Fighting Procedures: N/A

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid: Unknown
	Stable	X	

Incompatibility: None

Hazardous Decomposition or Byproducts: Sulfur oxides and bromides

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

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? No Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Acute eye contact: may cause irritation

Carcinogenicity: None | NTP? No data | IARC Monographs? No data | OSHA Regulation? No

Signs and Symptoms of Exposure: May cause skin or eye irritation

Medical Conditions Generally Aggravated by Exposure: None reported

Emergency First Aid Procedures: Rinse contacted areas 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/wipe spill area. Rinse with water.

Waste Disposal Method: Can be disposed in the trash or down the sink

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact

Other Precautions: None

Section VIII - Control Measures

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

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

Protective Gloves: Yes | Eye Protection: Splash prof 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): Phenol Red

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

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

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

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

Date Prepared: **07/01/03**

Signature of Preparer (optional)

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard. CAS # 7114-03-6				

Section III - Physical/Chemical Characteristics

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

Solubility in Water: Soluble

Appearance and Odor: Red color, liquid, no odor

Section IV - Physical/Chemical Characteristics

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

Extinguishing Media: N/A

Special Fire Fighting Procedures: N/A

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	Unknown

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? No Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Acute eye contact: may cause irritation

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
None	No data	No data	No

Signs and Symptoms of Exposure: May cause skin or eye irritation

Medical Conditions Generally Aggravated by Exposure: None reported

Emergency First Aid Procedures: Rinse contacted areas 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/wipe spill area. Rinse with water.

Waste Disposal Method: Can be disposed in the trash or down the sink

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact

Other Precautions: None

Section VIII - Control Measures

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

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

Protective Gloves: Yes

Eye Protection: Splash prof 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): Xylene Cyanol

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

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

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

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

Date Prepared: **07/01/03**

Signature of Preparer (optional)

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard. CAS # 2650-17-1				

Section III - Physical/Chemical Characteristics

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

Solubility in Water: Soluble

Appearance and Odor: _____ color, liquid, no odor

Section IV - Physical/Chemical Characteristics

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

Extinguishing Media: N/A

Special Fire Fighting Procedures: N/A

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	Unknown

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? No Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Acute eye contact: may cause irritation

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
None	No data	No data	No

Signs and Symptoms of Exposure: May cause skin or eye irritation

Medical Conditions Generally Aggravated by Exposure: None reported

Emergency First Aid Procedures: Rinse contacted areas 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/wipe spill area. Rinse with water.

Waste Disposal Method: Can be disposed in the trash or down the sink

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact

Other Precautions: None

Section VIII - Control Measures

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

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

Protective Gloves: Yes

Eye Protection: Splash prof 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): Bromophenol Blue

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

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

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

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

Date Prepared: **07/01/03**

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard. CAS # 62625-28-9				

Section III - Physical/Chemical Characteristics

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

Solubility in Water: Soluble

Appearance and Odor: Blue color, liquid, no odor

Section IV - Physical/Chemical Characteristics

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

Extinguishing Media: N/A

Special Fire Fighting Procedures: N/A

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	Unknown

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? No Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Acute eye contact: may cause irritation

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
None	No data	No data	No

Signs and Symptoms of Exposure: May cause skin or eye irritation

Medical Conditions Generally Aggravated by Exposure: None reported

Emergency First Aid Procedures: Rinse contacted areas 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/wipe spill area. Rinse with water.

Waste Disposal Method: Can be disposed in the trash or down the sink

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact

Other Precautions: None

Section VIII - Control Measures

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

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

Protective Gloves: Yes

Eye Protection: Splash prof 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): Methyl Orange

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

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

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

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

Date Prepared: **07/01/03**

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard. CAS # 547-58-0				

Section III - Physical/Chemical Characteristics

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

Solubility in Water: Soluble

Appearance and Odor: Yellow-orange color, liquid, no odor

Section IV - Physical/Chemical Characteristics

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

Extinguishing Media: N/A

Special Fire Fighting Procedures: N/A

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	Unknown

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? No Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Acute eye contact: may cause irritation

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
None	No data	No data	No

Signs and Symptoms of Exposure: May cause skin or eye irritation

Medical Conditions Generally Aggravated by Exposure: None reported

Emergency First Aid Procedures: Rinse contacted areas 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/wipe spill area. Rinse with water.

Waste Disposal Method: Can be disposed in the trash or down the sink

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact

Other Precautions: None

Section VIII - Control Measures

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

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

Protective Gloves: Yes

Eye Protection: Splash prof goggles

Other Protective Clothing or Equipment: None required

Work/Hygienic Practices: Avoid eye and skin contact