EDVOTEK®

Sci-On® Biology

The Biotechnology Education Company ®

S-53

EDVO-Kit #

The Mystery of the Crooked Cell

Storage:
Store the entire experiment at room temperature.

EXPERIMENT CONTENT OBJECTIVE

In this experiment, students will gain an understanding of the effect of mutations in health and disease, specifically as it relates to sickle cell anemia.

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

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INSTRUCTOR'S GUIDELINES

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

Experiment Components

Storage:
Store this experiment at room temperature.

This experiment is designed for 10 groups.

Contents

A Normal Hemoglobin control
B Sickle Hemoglobin control
C Carrier Hemoglobin control
D Patient #1 Hemoglobin
E Patient #2 Hemoglobin

Transfer pipets
UltraSpec-Agarose™
50x Electrophoresis Buffer
Practice Gel Loading Solution

None of the experiment components have been prepared from human sources. Simulated DNA samples are non-toxic, water-based dyes.

Experiment Requirements

Experiment Requirements

- Electrophoresis Apparatus, M-12 or equivalent
- D.C. Power Supply
- Heat Source
- 500 ml Beaker or Flask
- Hot Gloves
- Distilled Water (used to make buffer solutions)
- Balance
- Automatic Micropipet and tips (optional)

Background Information

A single nucleotide change in the DNA sequence of an important gene can affect health and disease. A large number of genetic diseases are identified where such changes have been correlated to changes in single nucleotides. More recently, mutations in oncogenes and tumor suppressor genes such as p53, have been associated with lung, colon and breast cancer. Other mutations in genes such as the BRCA 1 and II genes have been identified as specific markers with good potential as diagnostic tools for breast cancer.

Human genetics follows the basic findings of the Augustine monk, Gregor Mendel, who studied plant genetics in the mid-1800's. Mendelian genetics, which predicts traits inherited by offspring, is based on the inheritance of two alleles, or forms of the gene. These two alleles are inherited one from each parent. Alleles, and corresponding traits, can be either dominant or recessive. When a dominant allele is inherited, the trait coded by that allele will be apparent in the offspring. The presence of a dominant allele will, in effect, mask the trait coded by the recessive allele. To observe a recessive trait, it is required that both parental alleles be the recessive type. If both alleles are the same type, either both recessive or both dominant, the individual is said to be homozygous with respect to that trait. If an individual has one dominant and one recessive, the individual is said to be heterozygous for that trait.

	T	t
T	TT	T t
t	T t	t t

Figure 1

Genotype:

1/4	TT
1/2	Tt
1/4	tt

Phenotype:

3/4	dominant
1/4	recessive

Mendelian inheritance can be demonstrated with a 2 x 2 matrix, as shown in Figure 1. Parental alleles are placed on the sides of the matrix, and the genotype (what is genetically inherited) and phenotype (the way we look) of the offspring can be predicted. By convention, the dominant allele is denoted by an uppercase letter and the recessive allele by a lowercase letter. For example, assuming both parents each carry one dominant allele and one recessive allele, we can predict that 3/4 of their children will have the dominant phenotype and 1/4 of their children will have the recessive phenotype. Genotypically, 1/4 of the children will carry two dominant alleles; 1/2 of the children will carry one dominant and one recessive allele, and 1/4 will carry two recessive alleles. These estimates would be observed if there are a large number of offspring from two parents, as in the case of insects or plants.

Hemoglobin, which is present in red blood cells, is the carrier of oxygen to cells in the body. In capillaries carbon dioxide, which is a by product of metabolism, enters red cells and is converted to carbonic acid. The acidic pH reduces the affinity of oxygen binding to hemoglobin resulting in the release of oxygen in cells. Likewise when the bound carbon dioxide is released from red cells in the lungs there is an increase in pH which favors the binding of oxygen to hemoglobin. In individuals who suffer from certain blood diseases such as sickle cell anemia, the binding and subsequent transport of oxygen is compromised due to a single nucleotide mutation. This results in a deficiency of oxygen and carbon dioxide

Background Information

exchange in the patient. In sickle cell anemia patients, the substitution of the polar side chain (Glu) with a nonpolar hydrophobic side chain (Val) results in the polymerization of the unoxygenated form and subsequent precipitation of such polymers in red blood cells. The precipitation gives red blood cells a sickle shape due to the lack of diffusion through capillaries.

Each person has two copies of the gene of hemoglobin. Normal hemoglobin is referred to as Hemoglobin A. The letters AA are used to indicate that both hemoglobin genes are normal. The gene that causes sickle cell anemia is referred to as Hemoglobin S. There are three possible combinations of the genes for hemoglobin:

AA Individual is homozygous for the Hemoglobin A gene. So, both copies of hemoglobin code for normal hemoglobin and the person does not have the disease.

AS Individual is heterozygous. One copy of hemoglobin codes for normal hemoglobin and the other copy of the gene codes for sickled hemoglobin. This person does not have the disease and will not develop it later in life.

SS Individual is homozygous for the sickled hemoglobin S gene. So, both copies of hemoglobin code for diseased hemoglobin. This person suffers from sickled cell anemia.

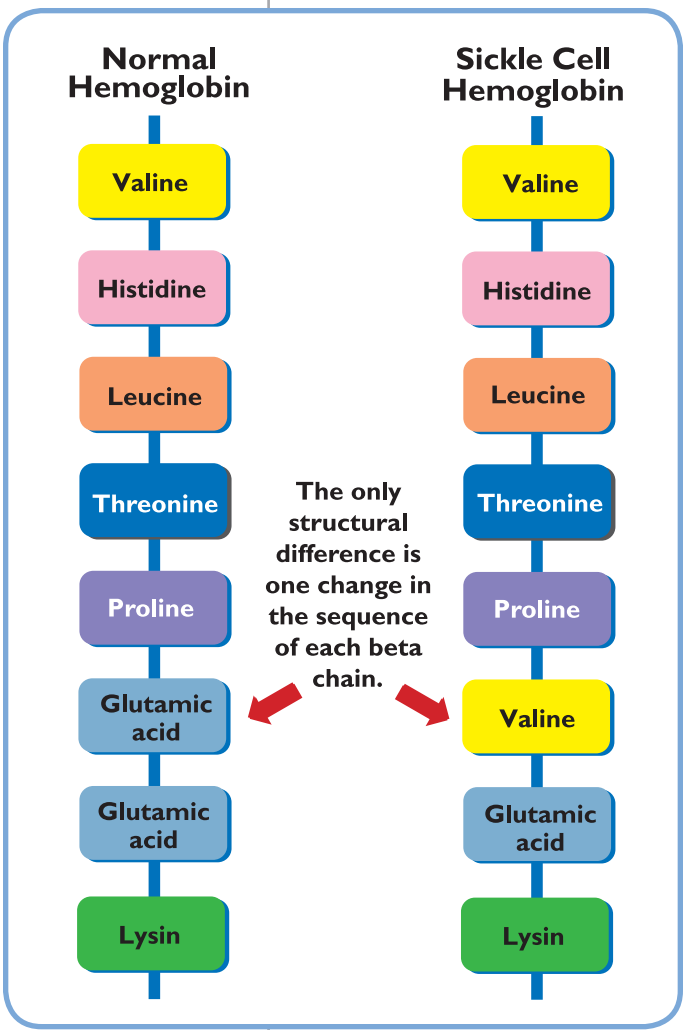
The irregularly shaped blood cells lead to a cascade of symptoms. The sickle-shaped blood cells die prematurely, resulting in anemia and the production of excess bilirubin (a yellow pigment resulting from the breakdown of hemoglobin). Jaundice often results when the liver cannot metabolize bilirubin fast enough. Infection, dehydration, overexertion, high altitude, chills, or cold weather can bring on a sickling episode, or crisis. Sometimes there is no apparent precipitating factor. People with sickle cell disease are susceptible to fevers and infection.

Blood disease such as sickle cell anemia and β-thalassemias are attributed to various point mutations or other translational product aberrations. Almost 400 different hemoglobin (Hb) variants of known structure have been identified. The early recognized variants were historically assigned alphabetical initials based sequence of discovery or hematologic features.

In the United States, sickle cell anemia is of special interest since it is estimated that 8% of African Americans are carriers of the sickle trait. It is of interest to note that heterozygous individuals for Hb S have a high resistance to the malaria parasite, part of whose life cycle is spent in red blood cells. Historically, sickle cell anemia has provided a selective advantage in some regions of the world such as parts of Africa. This can also explain the reason for the high frequency of this homozygous gene amongst African Americans.

Background Information

Hemoglobin is made up of two a chains and two β chains. The gene where the α is located is on the short arm of chromosome 16, while the β-globin gene cluster is on the short arm of chromosome 11. In addition to the adult form of Hb encoded within the β Hb cluster are the Hb forms that substitute for the adult β Hb during the various stages of development. Hemoglobin S (Hb S) is the variant form of the normal adult hemoglobin A (Hb A) in which an amino acid substitution occurs in the β polypeptide. The amino acid substitution is that of Valine (Val) in Hb S for the glutamic acid (Glu) normal Hb A hemoglobin (Figure 2). This significant finding was reported in 1957 by Vernon Ingram who was able to determine this amino acid substitution using peptide mapping analysis. These procedures are tedious and difficult. It should be noted that this predates recombinant DNA technology.



The single base mutation is an A to T in the triplet codon of the amino acid residue number 6 from the amino acid end in the beta chain. This change introduces an amino acid with a polar (neutral) side chain valine instead of the acidic (negative) residue and changes the property of the hemoglobin molecule. This substitution changes the electrophoretic mobility of Hb S compared to Hb A. At slightly basic pH, such as 8.4, Hb S will be relatively more positive than Hb A and therefore will travel slower towards the positive (anode) electrode. This change in mobility is used as a diagnostic test of the presence of Hb S.

With the advent of biotechnology, parental or fetal DNA from cells obtained from amniocentesis can now be analyzed with a high degree of accuracy. A few cells can provide sufficient DNA to be amplified using Polymerase Chain Reaction (PCR). Alternative methods can include growing cells in culture to yield sufficient DNA for analysis.

Background Information

The basis of the test is the recognition of specific palindromic sequences in DNA by restriction enzymes. In the normal β globin gene, the sequence of nucleotides that specifies amino acids 5, 6 and 7 (Pro-Glu-Glu) are CCT- GAG-GAG (see figure 2). The point mutation in codon 6 converting the A to T changes the sequence to CCT-GTG-GAG. The palindrome recognition site of the restriction enzyme *Mst* II is CCTNAGG, where N can be any of the four nucleotides. Close examination of the sequence shows that *Mst* II will recognize the normal β globin CCT-GAG-G where N is a G, but not the mutated form.

Experiment Overview

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

In this experiment, students will gain an understanding of the effect of mutations in health and disease, specifically as it relates to sickle cell anemia.

MATERIALS FOR THE EXPERIMENT

Each Lab Group should have the following materials:

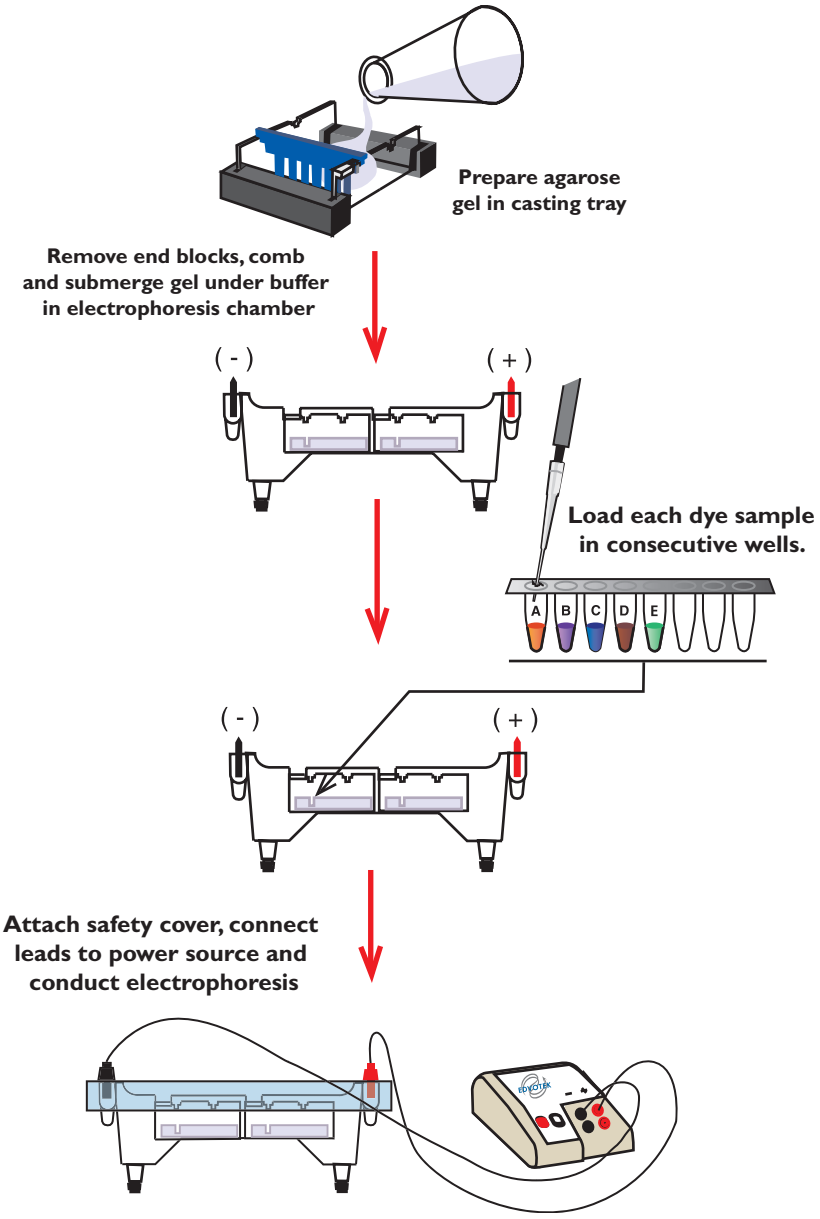
Activity One

- 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 - D) representing hemoglobin
- Sample delivery instrument
  - Automatic micropipet and tips, or
  - Transfer pipet and beaker of distilled water

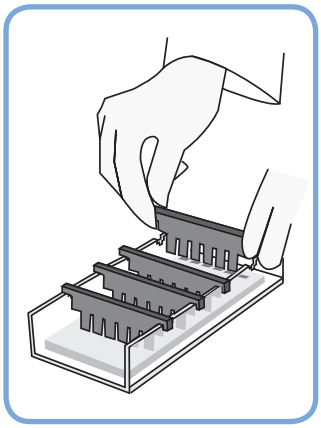
Experiment Overview



Activity One - 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.

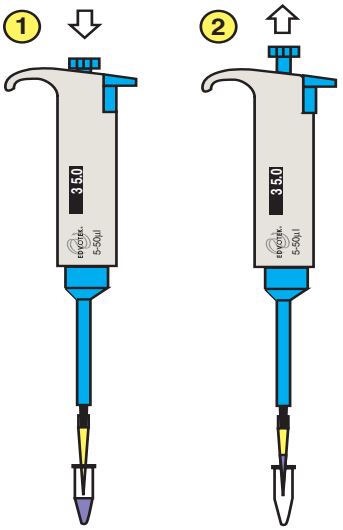
Note: The agarose gel is sometimes called a "submarine gel" because it is submerged under buffer for sample loading and electrophoretic separation.

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 15 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 practicing gel loading in the electrophoresis chamber, the practice gel loading solution will become diluted in the buffer in the apparatus. It will not interfere with the experiment, so it is not necessary to prepare fresh buffer.

Activity One - Practice Gel Loading

SAMPLE DELIVERY WITH VARIABLE AUTOMATIC MICROPIPETS:



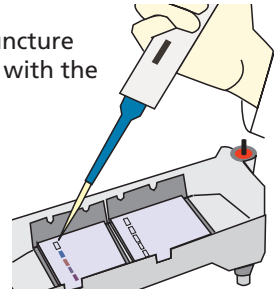
1. Set the micropipet to the appropriate volume and place a clean tip on the micropipetor.

   Press the top button down to the first stop. then immerse the tip into the sample.

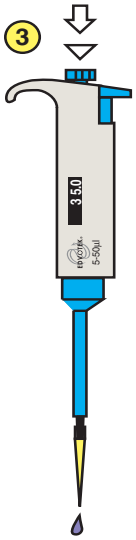
2. Once the tip is immersed in the sample, release the button slowly to draw sample into the tip.

3A. Position the pipet tip over the well.

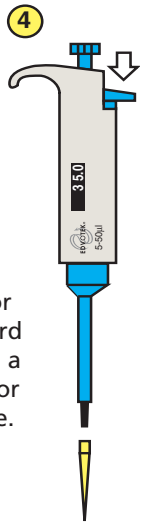
Be careful not to puncture or damage the well with the pipet tip.



3B. Deliver the sample by pressing the button to the first stop - then empty the entire contents of the tip by pressing to the second stop.



3C. After delivering the sample, do not release the top button until the tip is out of the buffer.



4. Press the ejector button to discard the tip. Obtain a new clean tip for the next sample.

Activity One - Practice Gel Loading

SAMPLE DELIVERY WITH PLASTIC TRANSFER PIPETS:

1. Gently squeeze the pipet stem to slowly
   The sample should remain in the lower p

   If the sample is overdrawn and becomes
   tap until the sample moves down in
   the pipet. Eject it back into the tube. Tr

2. While holding the pipet tip above the sa
   squeeze until the sample is nearly at the
   pipet tip.

3. Place the pipet tip in the electrophoresis
   rectly above barely inside the sample we

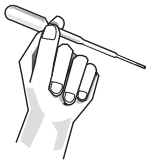
   Avoid placing the pipet tip all the way inside the well - this will minimize the chances of inadvertently piercing the bottom of the well.

4. MAINTAIN STEADY PRESSURE on the pipet stem to prevent buffer from being drawn in and diluting the sample.

5. Slowly squeeze to eject the sample. Stop squeezing when the well is about half full. Put any remaining sample in the pipet back into the sample tube.

6. Rinse the pipet with distilled water before obtaining the next sample for gel loading.

To control the delivery of small sample volumes with transfer pipets, gently squeeze the pipet stem, instead of the bulb.



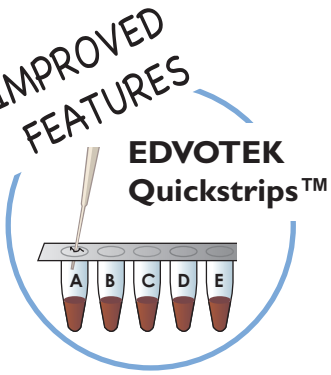
Activity Two - Conducting Agarose Gel Electrophoresis

ELECTROPHORESIS SAMPLES

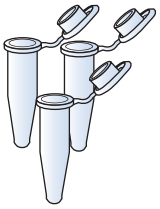
Samples in EDVOTEK Series 100 and S-series electrophoresis experiments are packaged in one of two different formats:

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.

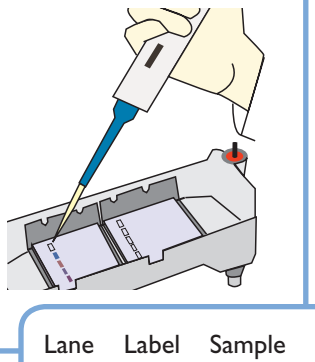


Quickstrips patent pending



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 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 - E into the wells in consecutive order. The amount of sample that should be loaded is 15 µl.

Lane	Label	Sample
1	A	Normal Hemoglobin control
2	B	Sickle Hemoglobin control
3	C	Carrier Hemoglobin control
4	D	Patient #1 Hemoglobin
5	E	Patient #2 Hemoglobin

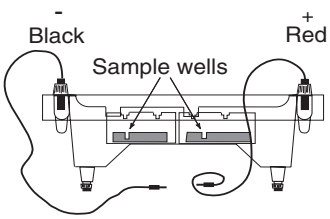
Activity Two - Conducting Agarose Gel Electrophoresis

RUNNING THE GEL

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

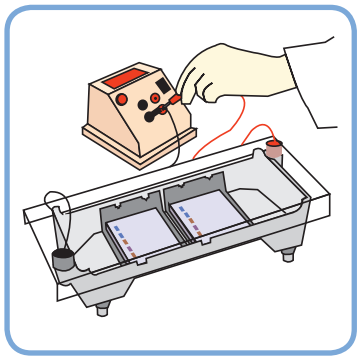
Reminders:

During electrophoresis, the samples will migrate through the agarose gel towards the positive electrode. Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.



the negative and positive color-coded indicators on the cover and us 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.

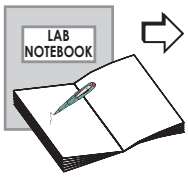
8. After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads and remove the cover.

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-53, 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.

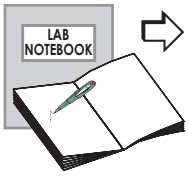
Critical Thinking and Hypothesis Development



Record the following in your Laboratory Notebook or on a separate sheet of paper:

1. Based on the evidence obtained from analysis of the gel, which patient has the sickle cell trait? Explain.
2. What is the variable in this experiment?
3. What would you change in the experiment if you had to do it over again?
4. Write a hypothesis that would reflect these changes.

Study Questions



Record the answers to the following Study Questions in your Laboratory Notebook or on a separate sheet of paper, as instructed by your teacher:

1. Why is it important to position the sample wells near the negative electrode?
2. Why is it important to use a new pipet or wash the pipet between uses?
3. How will you be able to tell which patient has the sickle cell trait?
4. Explain what happens to patients afflicted with sickle cell anemia?
5. What are the possible gene combinations for hemoglobin?

Notes:

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).

EDVO-TECH SERVICE

1-800-EDVOTEK
(1-800-338-6835)

Mon - Fri 9am - 6pm EST

Technical Service Department

Mon. - Fri.
9:00 am to 6:00 pm EST

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

Instructor's Notes

SUGGESTIONS FOR LESSON PLAN CONTENT

This lesson plan outline, written by a teacher, can be used as a guideline to fit your specific classroom experience. The connections to the **National Content and Skills Standards** appear on pages that follow.

1. This experiment can be presented as a hypothetical medical diagnostics case.

   Alternatively, have students write a creative scenario that is based on the analysis of the gel.

2. Using electrophoresis to separate fragments is used in areas other than medical diagnostics. Have students conduct an "on-line" search to see how this procedure is used in the following cases:

   - Forensic Science.
   - Identifying men and women killed in service.
   - Determining paternity

3. Discuss the importance of being meticulous in the collection of patient hemoglobin samples.

4. From the vocabulary list of words below, have students write application sentences about each:

   Hemoglobin Restriction Enzyme Sickle Cell Anemia
   Electrophoresis Mutation Genotype Phenotype

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 variables
   - Predicting experimental outcomes
   - Recording results in a concise and accurate manner
   - Drawing valid interpretations of results
   - Formulating alternative explanations

Instructor's Notes

ELECTROPHORESIS ANALYSIS OF SIMULATED DNA SAMPLES

DAY ONE

1. Have gels prepared in advance and hand out lab instructions.

2. The electrophoresis samples in this experiment are packaged in new pre-aliquoted Quickstrip™ tubes. Several Quickstrips are connected in a block sealed with foil top cover. The sample block can easily be separated by scoring and tearing, or cut with scissors into strips of connected tubes. Each strip of connected tubes comprise a complete set of samples for each gel.

3. Have students practice loading sample wells before doing the experiment.
   - If using micropipets, review proper use.
   - Thoroughly rinse wells before proceeding with experiment.
   - Remind students to keep track of what samples were loaded into which wells.

5. Clean up and answer student questions.

DAY TWO

1. Conduct the actual electrophoresis experiment.
   - Have students load the experiment samples.
   - Remind students to keep track of what samples were loaded into which wells.

2. At the end of the electrophoresis run, have students view and sketch results.

3. Have students answer a list of Study Questions and go over answers to the Study Questions.

OPTIONAL ACTIVITY FOR THE STUDENTS:

- Provide students with a list of topics for further research (library or newspaper research, written report, etc.).

- Set a date for students to report their research results.

Connections to National Content Standards

1. Students will develop abilities necessary to do scientific inquiry.
   - Student questions will be answered through conduction of 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 variable.
   - Students will predict experimental outcomes.
   - Students will formulate explanations from evidence.
   - 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.
   - Students will understand how different sizes of fragments are separated by agarose gel electrophoresis.

4. Students will develop an understanding of the function of restriction enzymes.
   - Students will understand that restriction enzymes are endonucleases which catalyze the cleavage of bonds within both strands of DNA.
   - Students will understand that points of cleavage occur in or near very specific sequences of bases called recognition sites.
   - Students will understand that the number of bases in a recognition site and the distance between the recognition sites determines the size of the DNA fragment produced.

Connections to National Skill Standards

In this experiment 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 units.
2. Accurately load and run an agarose gel.
3. Make careful observations and record results.
4. Perform a medical diagnostics procedure.
5. Compare and evaluate hemoglobin patterns.

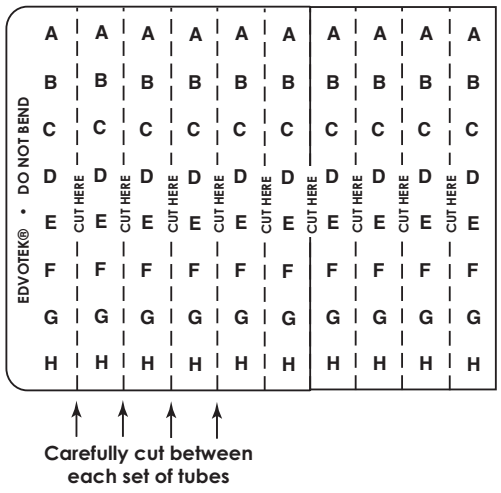
Instructor's Guidelines

Preparations for the Experiment

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-E in the block of samples.

   Note: In this experiment, tubes F - H are empty.

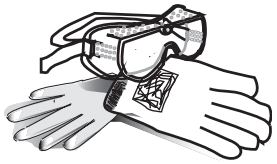
2. Cut carefully through the foil between the rows of samples. Do not cut or puncture the foil covering the top of the sample tubes.

3. Each group will require one strip of samples.

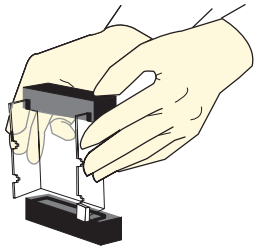
4. Remind students to tap the foil or tubes before gel loading to ensure that all of the sample is at the bottom of the tube.

Preparations for the Experiment

This experiment requires an 0.8% agarose gel. Agarose gels can be prepared before the laboratory period and stored under buffer. The simulated DNA samples (dyes) are ready-to-load for electrophoresis. Agarose gels can be prepared individually, or a batch preparation of agarose gel solution can be prepared to cast several gels at the same time. See page 24 for batch gel preparation instructions.



Wear gloves and safety goggles



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.

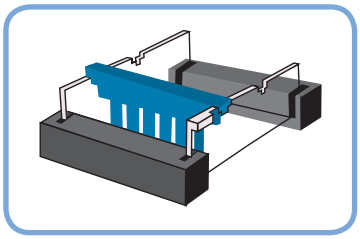
   A. Using Rubber dams:

   - 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 t end of the gel bed. Make sure the comb bed.



Preparations for the Experiment

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.

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

Preparations for the Experiment

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.

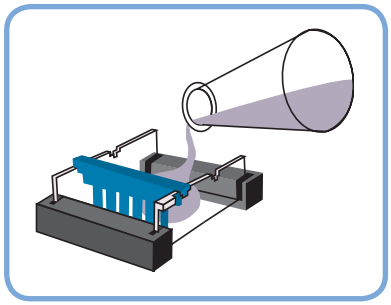
Cool the agarose to 55˚C

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.



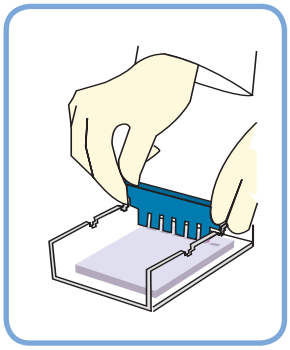
8. Seal the interface of the gel bed and tape to prevent the agarose solution from leaking.
   - Use a transfer pipet to deposit a small amount of cooled agarose to both inside ends of the bed.
   - Wait approximately 1 minute for the agarose to solidify.
9. Pour the cooled agarose solution into the 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.

Preparations for the Experiment

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

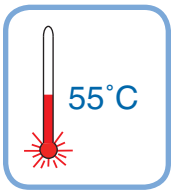
EDVOTEK Model #	Concentrated Buffer (50x) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
M6+	6		294		300
M12	8		392		400
M36 (blue)	10		490		500
M36 (clear)	20		980		1000

Preparations for the Experiment

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.



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.

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

Preparations for the Experiment

ELECTROPHORESIS TIME AND VOLTAGE

Your schedule will dictate the length of time samples will be separated by electrophoresis. In general, longer electrophoretic runs will increase the separation between fragments of similar size.

Because this experiment involves the electrophoresis of dyes, it can be easily monitored visually. Follow general guidelines as presented in Table C, but monitor the electrophoresis to make sure that the dyes do not migrate off the end of the gel.

Table C Time and Voltage

Electrophoresis of Dyes

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

If you don't find answers to your questions in this section, call our

Technical Service Department

EDVO-TECH SERVICE
1-800-EDVOTEK
(1-800-338-6835)
Mon - Fri 9am - 6pm EST

Mon. - Fri.
9:00 am to 6:00 pm EST

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

Please have the following information:

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

www.edvotek.com

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

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.

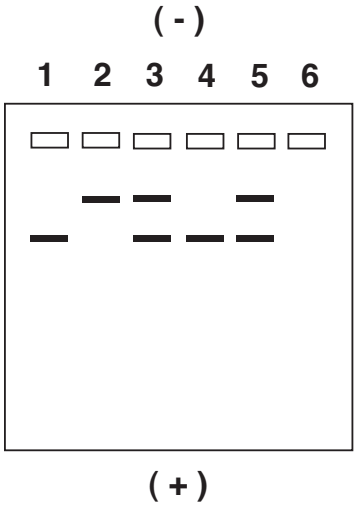
3. Electrophoresis should be terminated when the dyes have moved 3 to 4 centimeters from the wells and before it moves off the gel.

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.

Experiment Results and Analysis



Idealized results are shown in the figure at left. Actual results will yield bands of varying intensity. The idealized schematic shows the relative positions of the bands, but are not depicted to scale.

Lane	Label	Sample
1	A	Normal Hemoglobin control
2	B	Sickle Hemoglobin control
3	C	Carrier Hemoglobin control
4	D	Patient #1 Hemoglobin
5	E	Patient #2 Hemoglobin

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_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	Unstable		Conditions to Avoid
	Stable	X	None

Incompatibility: None

Hazardous Decomposition or Byproducts: Sulfur oxides, and bromides

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

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): Food dye

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	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	Unstable		Conditions to Avoid
	Stable	X	Unknown

Incompatibility: None

Hazardous Decomposition or Byproducts: Sulfur oxides and bromides

Hazardous Polymerization	May Occur		Conditions to Avoid
	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: 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