



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.

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Experiment Components

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

Storage:
Store entire experiment at room temperature.

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.

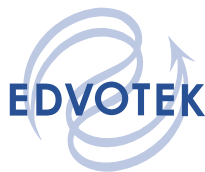
Genetic diseases can be caused by a single base substitution, as in sickle cell anemia, or a rearranged gene that has deleted (truncated) sequences, such as in certain cancers. Environmental factors, such as carcinogens or certain viruses, can contribute to non-inherited gene modifications.

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



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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 direction (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.

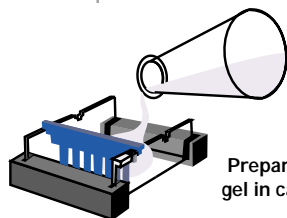
In an additional activity, migration distances of the dyes representing genes can be measured and plotted on semi-log graph paper. Dyes with assigned sizes (standard dyes) are used to plot a standard curve. The sizes of the various dyes ("genes") can be obtained by measuring their mobility and determining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

Experiment Overview

Experiment Procedures

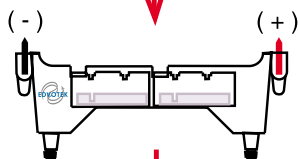
BEFORE YOU START THE EXPERIMENT

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

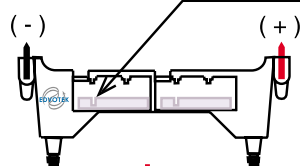
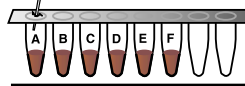


Prepare agarose gel in casting tray

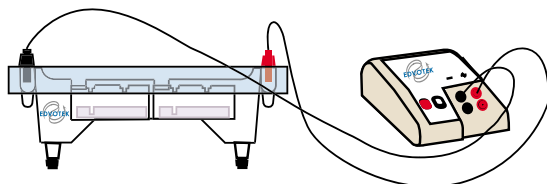
Remove end blocks, comb and submerge gel under buffer in electrophoresis chamber



Load each dye sample in consecutive wells.



Attach safety cover, connect leads to power source and conduct electrophoresis

**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.



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Activity One - Agarose Gel Preparation and Practice Gel Loading

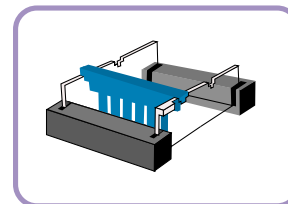
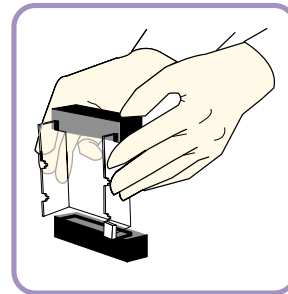


LABORATORY SAFETY

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.



Activity One - Agarose Gel Preparation and Practice Gel Loading

Experiment Procedures

CASTING AGAROSE GELS

- 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

- Swirl the mixture to disperse clumps of agarose powder.
- With a marking pen, indicate the level of the solution volume on the outside of the flask.
- Heat the mixture to dissolve the agarose powder. The final solution should appear clear (like water) without any undissolved particles.
 - 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.
 - 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.

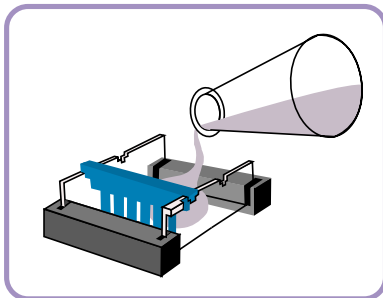
Check the solution carefully. If you see "crystal" particles, the agarose is not completely dissolved.

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

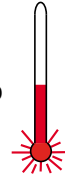


Activity One - Agarose Gel Preparation and Practice Gel Loading

- 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:

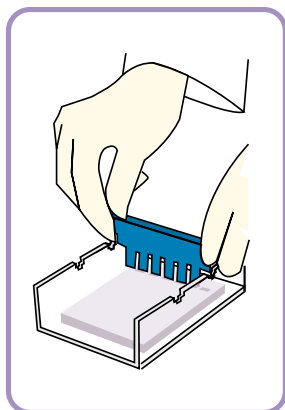
**If you are using rubber dams, go to step 9.
If you are using tape, continue with step 8.**

- 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.
- Pour the cooled agarose solution into the bed. Make sure the bed is on a level surface.
- 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



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.

PREPARING THE GEL FOR ELECTROPHORESIS

- 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.

- Remove the comb by slowly pulling straight up. Do this carefully and evenly to prevent tearing the sample wells.
- Place the gel (on its bed) into the electrophoresis chamber, properly oriented, centered and level on the platform.
- 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.

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

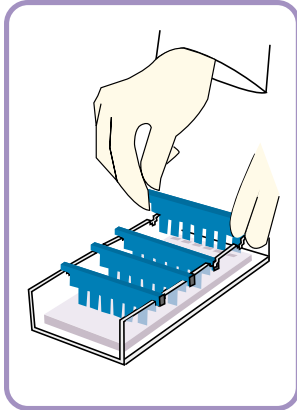
- Make sure the gel is completely covered with buffer.
- Proceed to loading the samples and conducting electrophoresis.



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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

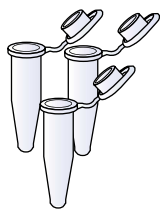
Experiment Procedures

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.



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.



Quickstrips
patent pending

LOADING THE SAMPLES

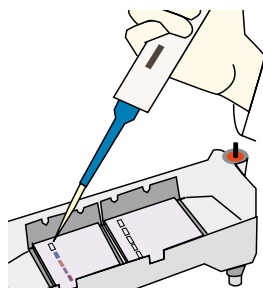
1. Check the Sample Volumes

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 - 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



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Activity Two - Conducting Agarose Gel Electrophoresis

RUNNING THE GEL

- 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.

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

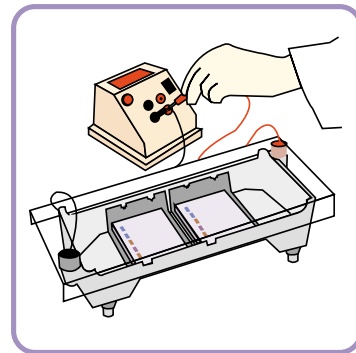


Table C Time and Voltage

Electrophoresis of Dyes

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

- After approximately 10 minutes, you will begin to see separation of the colored dyes.
- After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads and remove the cover.
- 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")

Quick Reference:

The Standard dyes have the following base pair equivalents.

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

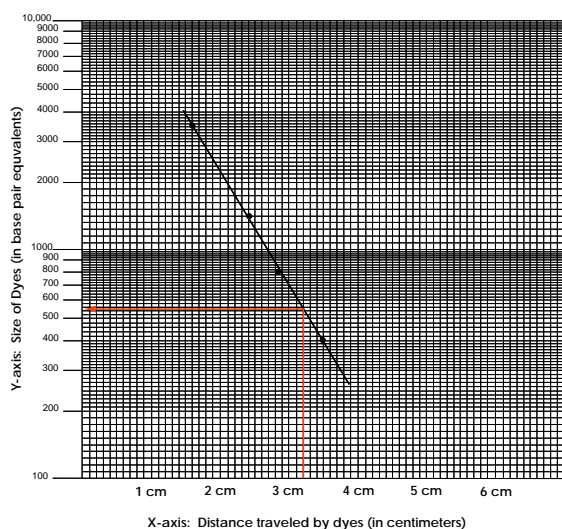
This exercise focuses on the first steps for mapping, which is used in the Human Genome Project. The assignment of sizes for the dyes separated by agarose gel electrophoresis can have $\pm 10\%$ margin of error. The sizes of the "unknowns" are extrapolated by their migration distances relative to the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

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

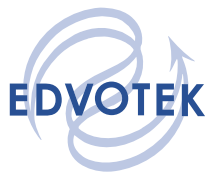
In each case, measure from the lower edge of the sample well to the lower end of each band. Record the distance traveled in centimeters (to the nearest millimeter).

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).



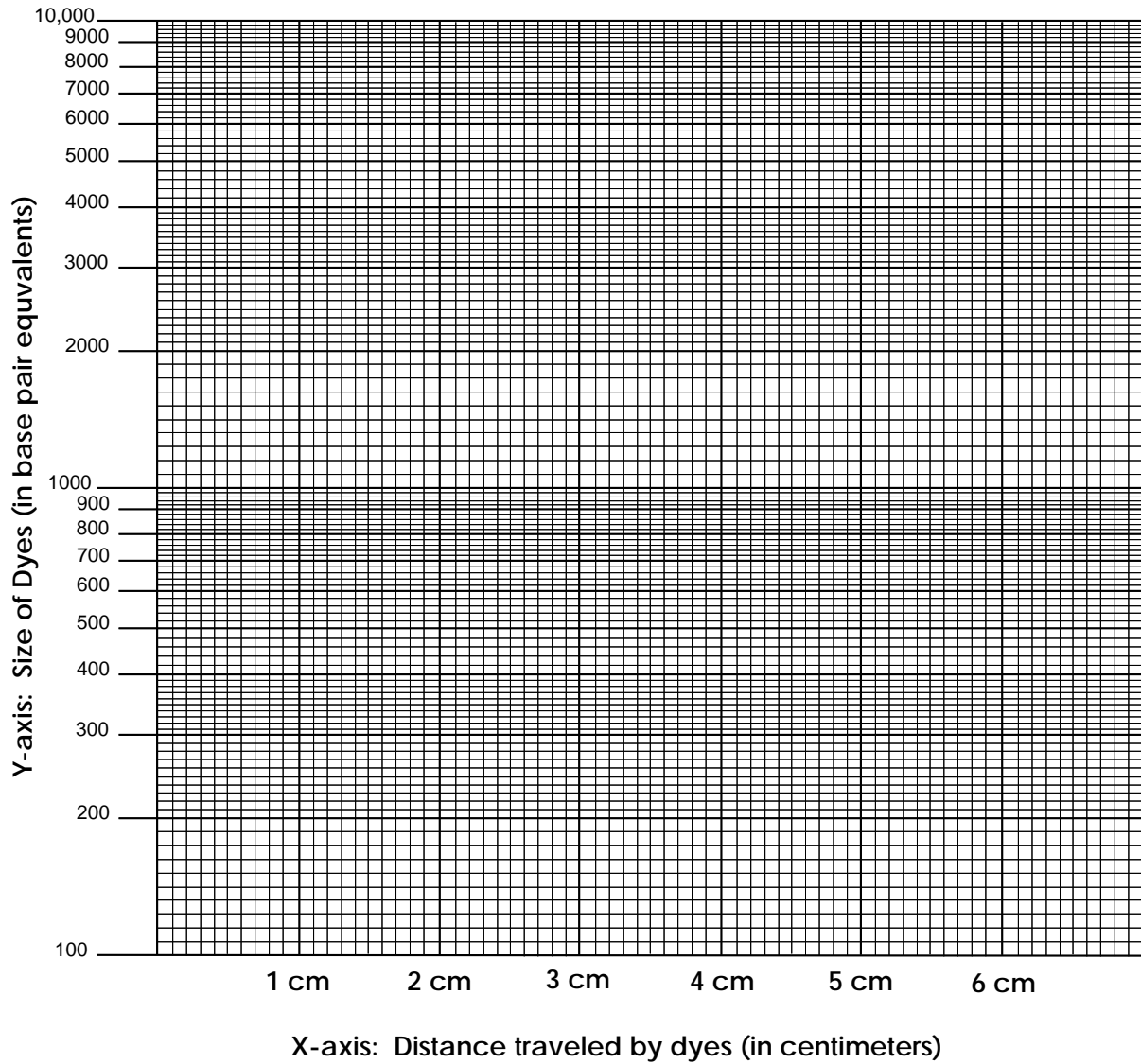
5. Measure the migration distance of each of the "unknown" dyes from samples B, C, D, E and F.
6. Using the graph of the Standard dyes below, plot and determine the sizes of each "unknown" dye.
 - A. Find the migration distance of the unknown dye on the x-axis, and draw a vertical line from that point 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.



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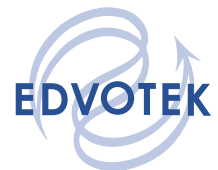
Activity Three - Size Determination of Dyes ("Genes")



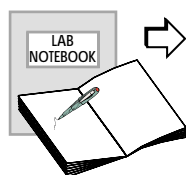
Experiment Procedures

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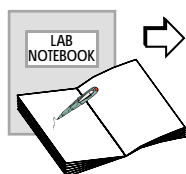
Critical Thinking and Hypothesis Development



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.

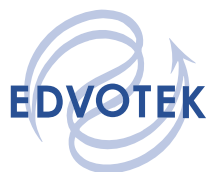
Study Questions



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.




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 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 ₂ O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data
Solubility in Water Insoluble - cold			
Appearance and Odor White powder, no odor			
Section IV - Physical/Chemical Characteristics N.D. = No data			
Flash Point (Method Used)	No data	Flammable Limits	LEL N.D. UEL 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			

 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 ₂ O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data
Solubility in Water 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)	No data	Flammable Limits	LEL N.D. UEL 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?			
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 environmental 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			

 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		Emergency Telephone Number (301) 251-5990	
Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, Zip Code) 14676 Rothgeb Drive Rockville, MD 20850		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 ₂ O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data
Solubility in Water Soluble			
Appearance and Odor Blue liquid, no odor			
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
	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			

 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		Emergency Telephone Number (301) 251-5990	
Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, Zip Code) 14676 Rothgeb Drive Rockville, MD 20850		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 ₂ O = 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
	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			

 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		Emergency Telephone Number (301) 251-5990	
Manufacturer's Name EDVOTEK, Inc.		Telephone Number for information (301) 251-5990	
Address (Number, Street, City, State, Zip Code) 14676 Rothgeb Drive Rockville, MD 20850		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 ₂ O = 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)	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
	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:	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			

 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		Emergency Telephone Number (301) 251-5990	
Manufacturer's Name EDVOTEK, Inc.		Telephone Number for information (301) 251-5990	
Address (Number, Street, City, State, Zip Code) 14676 Rothgeb Drive Rockville, MD 20850		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 ₂ O = 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)	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
	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:	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			

		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	
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 ₂ O = 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)	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
	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:	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			

		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	
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 ₂ O = 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
	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:	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			