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Experiment Components

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA.

Quick Reference:

After the restriction enzyme digestion of DNA samples, each group prepares a sample for agarose gel electrophoresis. The samples can be electrophoresed on one or more gels. This experiment contains enough reagents for five gels based upon the use of Horizontal gel electrophoresis apparatus, Model #M12. This experiment contains biologicals for 10 groups to perform restriction enzyme digestion of Lambda DNA with *Eco* RI, and electrophoresis reagents sufficient for five gels.

	Contents	Storage
Α	Lambda phage DNA	Freezer
B	Concentrated Restriction Enzyme Reaction Buffer	Freezer
С	EDVOTEK enzyme grade water	Freezer
D	<i>Eco</i> RI Dryzymes [™] endonuclease	Room temp with desiccant
Ε	Standard DNA Fragments	Freezer
F	Reconstitution Buffer F	Freezer
G	Reconstitution Buffer G	Freezer
	10x Gel Loading Solution Practice Gel Loading Solution UltraSpec-Agarose [™] powder 50x concentrated electrophoresis buffer Concentrated Methylene Blue Plus [™] stain DNA Blue InstaStain [™] 1 ml pipets 100 ml plastic graduated cylinder Transfer pipets Microtest tubes with attached caps Semi-log graph paper template	

UltraSpec-Agarose, Dryzymes, DNA Blue InstaStain and Methylene Blue Plus are all trademarks of EDVOTEK, Inc.

Requirements

- Horizontal Gel Electrophoresis Apparatus
- D.C. Power Supply
- Automatic Micropipets and tips
- Waterbath (37°C)
- Small plastic trays or large weigh boats for gel staining
- DNA Visualization System (visible light)
- Microwave, hot plate or burner
- Hot Gloves
- Pipet Pumps or bulbs
- 5 or 10 ml pipets
- 250 ml flasks or beakers
- 500 ml graduated cylinder
- Vinyl gloves and safety goggles
- Distilled or Deionized Water
- Ice
- Metric rulers

BACKGROUND INFORMATION

Cleavage of Lambda DNA with Eco RI Endonuclease

AN INTRODUCTION TO RESTRICTION ENZYMES

The discovery of restriction enzymes has ushered in a new era of molecular genetics. These enzymes give us the ability to cut DNA in a highly specific and reproducible way. This, in turn, has ushered in the area of molecular cloning, mapping and sequencing the fine genetic structure. These procedures, in turn, have made the Human Genome Project possible.

Restriction enzymes are endonucleases which catalyze the cleavage of the phosphate bonds within both strands of DNA. They require Mg⁺² for activity and generate a 5 prime (5') phosphate and a 3 prime (3) hydroxyl group at the point of cleavage. The distinguishing feature of restriction enzymes is that they only cut at very specific palindromic sequences of bases. Restriction enzymes are produced by many different species of bacteria (including blue-green algae). Over 1500 restriction enzymes have been discovered and catalogued. More recently, intron-coded yeast mitochondrial endonucleases have been discovered that also cut DNA. The recognition sequences for these enzymes are such that they yield very few cuts in DNA and promise to be important new biological reagents for the Human Genome Project.

Restriction enzymes are named according to the organism from which they are isolated. This is done by using the first letter of the genus followed by the first two letters of the species. Only certain strains or sub-strains of a particular species may produce restriction enzymes. The type of strain or substrain sometimes follows the species designation in the name. Finally, a Roman numeral is always used to designate one out of possibly several different restriction enzymes produced by the same organism or by different substrains of the same strain.

A restriction enzyme requires a specific double-stranded recognition sequence of nucleotide bases to cut DNA. Recognition sites are usually 4 to 8 base pairs in length. Cleavage occurs within or near the site. The cleavage positions are indicated by arrows. Recognition sites are frequently symmetrical, i.e., both DNA strands in the site have the same base sequence when read 5' to 3'. Such sequences are called palindromes. Consider the recognition site and cleavage pattern of Eco RI as an example.

> \downarrow 5'-GAATTC-3' 3'-CTTAAG-5' ↑

5'-G AATTC-3' 3-CTTAA G-5'

Restriction Enzyme	Organism
Bg/1	Bacillus globigii
Bam HI	Bacillus amyloliquefaciens H
Eco RI	Escherichia coli, strain RY 13
Eco RII	Escherichia coli, strain R 245
Hae III	Haemophilus aegyptius
Hind III	Haemophilus influenzae R _d

BACKGROUND INFORMATION

Background Information, continued

As you can see, *Eco* RI causes staggered cleavage of its site. The resulting ends of the DNA fragments are called "sticky" or "cohesive" because the single-stranded regions of the ends are complementary.

Some restriction enzymes, such as *Hae* III, introduce cuts that are opposite each other. This type of cleavage generates "blunt" ends.

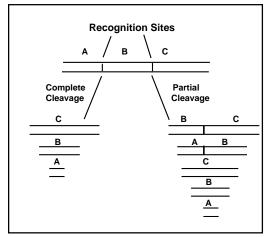
\checkmark		
5'-GGCC-3'	5'-GG	CC-3'
3'-CCGG-5'	3'-CC	GG-5'
\uparrow		

The recognition sites of some restriction enzymes contain variable base positions. For example, *Ava* I recognizes:

\downarrow	
5'-CPyCGPuG-3'	$(Py = pyrimidine = C \mathbf{or} T and$
3'-GPuGCPyC-5'	Pu = purine = G or A
Č↑	•

Keep in mind that A pairs with T and G pairs with C. Consequently, there are four possible sequences *Ava* I recognizes. Recognition sites of this type are called degenerate.

There are some recognition sites that are divided by a certain number of totally variable bases. For example, *Bgl* I recognizes:





 \downarrow 5'-GCCNNNNNGGC-3' (N = A, G, C <u>or</u> T) 3'-CGGNNNNNCCG-5'

There are 625 possible sequences *Bgl* I can cleave. The only bases the enzyme truly "recognizes" are the six G-C base pairs at the ends, which form a palindrome. In the case of *Bgl* I, these true recognition bases must always be separated by 5 base pairs of DNA; otherwise, the enzyme cannot properly interact with the DNA and cleave it. Recognition sites like that of *Bgl* I are called hyphenated sites.

In general, the longer the DNA molecule, the greater the probability that a given recognition site will occur. The probability of DNA digestion is directly proportional to the size of the enzyme

recognition palindrome. Thus, an enzyme that recognizes four nucleotides will cut DNA on average once every 256 base pairs, while an enzyme that recognizes five base pairs will cut DNA once every 1024 base pairs. Chromosomal DNA, which can contain billions of base pairs, has many more recognition sites than a plasmid DNA containing only a few thousand base pairs. However, very large DNA is difficult to isolate intact. During handling, it is randomly sheared to fragments in the range of 50,000 to 100,000 base pairs.

Background Information, continued

Quick Reference:

Standard DNA fragments, which were generated by restriction enzymes are provided in this experiment. A standard curve will be made on semi-log graph paper. The following are the Standard DNA fragment sizes length is expressed in base pairs.

23130	9416	6557	
4361	3000	2322	
2027	725	570	

BACKGROUND INFORMATION

Plasmids and many viral DNAs are circular molecules. If circular DNA contains one recognition site for a restriction enzyme, then it will open up to form a linear molecule when cleaved. By contrast, if a linear DNA molecule contains a single recognition site, it will be cleaved once to form 2 fragments. The size of the fragments produced depends on how far the sites are from each other. If a DNA molecule contains several recognition sites for a restriction enzyme, then under certain experimental conditions, it is possible that certain sites are cleaved and not others. These incompletely cleaved fragments of DNA are called partials. Partials can arise if low amounts of enzyme are used or the reaction is stopped after a short time. In reality, reactions containing partials also contain some molecules that have been completely cleaved.

Agarose gel electrophoresis is a powerful separation method frequently used to analyze DNA fragments generated by restriction enzymes. The gel consists of microscopic pores that act as a molecular sieve. Samples of DNA are loaded into wells made in the gel during casting. Direct current is then applied. Since DNA has a strong negative charge at neutral pH, it migrates through the gel towards the positive electrode during electrophoresis. Linear DNA molecules are separated according to their size. The smaller the linear fragment, the faster it migrates. If the size of two fragments are similar or identical, they will migrate together in the gel. This is called a doublet. If DNA is cleaved many times the wide range of fragments produced will appear as a smear after electrophoresis. Other forms of DNA, such as circular and superhelical, are separated in the gel according to their size and shape.

The Lambda DNA used in this experiment is isolated as a linear molecule from the *E. coli* bacteriophage lambda. It contains approximately 49,000 base pairs and has 5 recognition sites for *Eco* RI, and 7 for *Hind* III. The smaller fragments generated by a restriction enzyme, such as those generated by *Hind* III, may not be visible after agarose gel electrophoresis. Since there is less mass in the bands containing smaller fragments, they stain with less intensity and may be undetectable. Smaller fragments can also run off the gel. Stoichiometric cleavage of a pure sample of DNA results in equimolar amounts of fragments.

Lambda phage DNA contains 10-16 base single-stranded regions at the 5' and 3' terminus which are self-complementary, called <u>cos</u> ends. To properly resolve lambda phage DNA fragments, they must be heated to 65°C before loading onto the gel. For example, the 4361 and 23130 base pair fragments will hybridize at the "cos" sites, and the amount of the 4361 base pair fragment will be decreased and hard to visualize on the stained gel.

EXPERIMENTAL PROCEDURES

EXPERIMENT OBJECTIVE

The objective of this experiment module is to develop an understanding of restriction enzymes and agarose gel electrophoresis.

LABORATORY SAFETY

This experiment is designed for staining of DNA with Methylene Blue Plus[™] stain after electrophoresis. Methylene Blue Plus[™], which is included with this experiment, is formulated to provide optimal sensitivity for visualization when used in conjunction with EDVOTEK equipment and in accordance with specified experimental procedures. Optimal visualization is obtained by using a Visible Light Gel Visualization system. As with any biological stain, care should be taken when handling solutions or gels containing methylene blue.

Gloves and goggles should be worn when handling methylene blue staining reagents, and worn routinely throughout the experiment as good laboratory practice.



WEAR SAFETY GOGGLES AND GLOVES

EXPERIMENTAL PROCEDURES

Restriction Enzyme Digestion



AND GLOVES

Useful Hint!	
To transfer the samples from one tube to another, you may use an automatic pipet or the transfer pipets provided.	
To control the delivery of small sample volumes with transfer pipets, gently squeeze the pipet	

stem, instead of the bulb.

You should have two tubes of the reaction buffer and two tubes of DNA. Use one set of tubes consisting of the reaction buffer (tube "1") and the DNA (tube "2") for the control, and the other set for the digestion reaction as outlined below.

SETTING UP THE CONTROL

- 1. With a <u>FRESH</u> pipet tip or transfer pipet, transfer all of the reaction buffer (tube "1") to a tube containing DNA (tube "2").
- 2. Using the same pipet or tip, transfer the contents of tube 2 to the tube with water (tube "3").
- 3. Cap and label tube 3 "control" and put your group number or initials on it.

SETTING UP THE DIGESTION REACTION

- 4. With a <u>FRESH</u> pipet tip or transfer pipet, transfer all of the reaction buffer (tube "1") to the second tube containing DNA (tube "2").
- 5. Transfer all the contents of tube 2 to the tube with *Eco* RI (tube "4").
- 6. Cap and label tube 4 "reaction" and put your group number or initials on it.
- 7. Tap both the "Control" and "Reaction" tubes to mix.
- 8. Incubate both tubes in a 37°C water bath for 30-60 minutes.

AFTER THE 30-60 MINUTE INCUBATION:

9. Add 5 μ l of 10x gel loading solution to each tube.

If you are using a transfer pipet, add 1 drop to each tube.

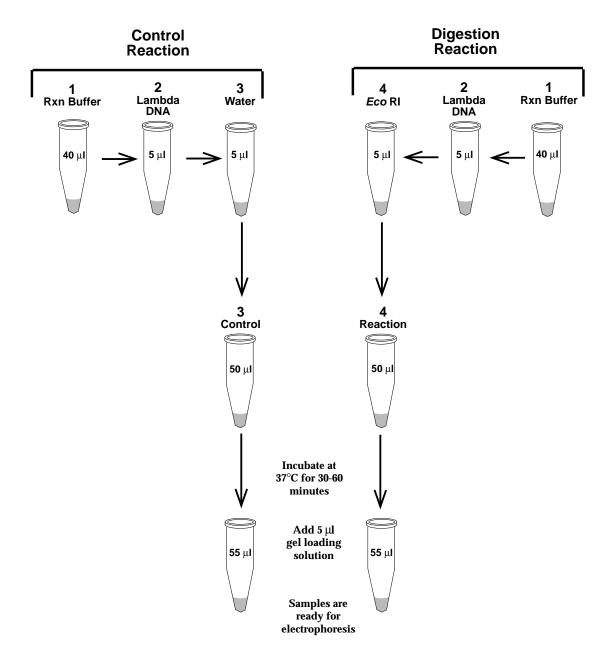
10. Cap the tubes and mix. The samples are ready for electrophoresis, or may be frozen for electrophoresis at a later time.

Table 1: Summary of Restriction Enzyme Digestion Reaction							
$Tube_{\rightarrow}$	1 Buffer	2 DNA	3 Water	4 Eco RI	Final Rxn Volume	37° C	10x Gel Load
Control	40 µl	5 μl	5 μl		50 μl	30-60 min	5 μl
Digestion Reaction	40 µl	5 μl		5 µl	50 µl	30-60 min	5 μl

EXPERIMENTAL PROCEDURES

Restriction Enzyme Digestion, continued

SCHEMATIC FLOW DIAGRAM OF RESTRICTION ENZYME DIGEST



Tube numbers correspond to number assignments in Table 1 (page 9)

EXPERIMENTAL PROCEDURES

Agarose Gel Preparation

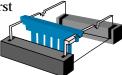
PREPARING THE GEL BED

Using 7 x 7 cm Gel Beds

- 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 sits firmly in contact with the sides and bottom of the bed.

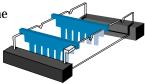


- B. Taping with labeling or masking tape:
 - With 3/4 inch wide tape, extend the tape over the sides and bottom edge of the bed.
 - Fold the extended edges of the tape back onto the sides and bottom. Press contact points firmly to form a good seal.
- 2. Place a well-former template (comb) in the first set of notches nearest the end of the gel bed. Make sure the comb sits firmly and evenly across the bed.



Using the 7 x 15 cm Gel Bed for Two Gels

- 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 sits 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 nearest the end of the gel bed. Place a second comb in the middle set of notches. Make sure the combs sit firmly and evenly across the bed.



EXPERIMENTAL PROCEDURES

Agarose Gel Preparation, continued

CASTING THE GEL

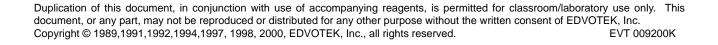
This experiment requires a 0.8% gel.

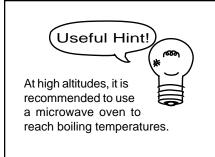
- 3. Use a 250 ml flask to prepare the diluted gel buffer.
 - With a 1 ml pipet, measure the buffer concentrate and add the distilled water as indicated in **Table A**.

Table A:Individual 0.8 % UltraSpec-Agarose™ Gels for staining with DNA Blue InstaStain™				
Size of EDVOTEK Casting Tray	Amt of Agarose +	Concentrated Buffer (50x)	Distilled + Water :	Total = Volume
7 x 7 cm	0.24 gm	0.6 ml	29.4 ml	30 ml
7 x 15 cm	0.48 gm	1.2 ml	58.8 ml	60 ml
10.5 x 14 cm	0.8 gm	2.0 ml	98.0 ml	100 ml

- 4. Add the required amount of agarose powder. Swirl to disperse clumps.
- 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 be clear (like water) without any undissolved particles.
 - A. Microwave method:
 - Cover 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 or burner method:
 - Cover the flask with foil to prevent excess evaporation.
 - Heat the mixture to boiling over a burner with occasional swirling. Boil until all the agarose is completely dissolved.
- 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.







EXPERIMENTAL PROCEDURES

Agarose Gel Preparation, continued



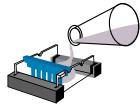
DO NOT POUR BOILING HOT AGAROSE INTO THE GEL BED. Hot agarose solution may irreversibly warp the bed.

Useful Hint! Step 11: Be careful not to damage or tear the gel when removing rubber dams. A thin plastic knife or spatula can be inserted between the gel and the dams to break possible surface tension.

After the gel is cooled to 55°C:

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

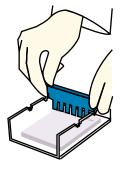
- 8. Seal the interface of the gel bed and tape to prevent the agarose solution from leaking.
 - Use a transfer pipet to deposit a small amount of cooled agarose to both inside ends of the bed.
 - Wait approximately 1 minute for the agarose to solidify.
- 9. Pour the cooled agarose solution into the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

PREPARING THE GEL FOR ELECTROPHORESIS

- 11. After the gel is completely solidified, carefully and slowly remove the rubber dams or tape.
- 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 (see guidelines presented in Table B).

Table B:	Electrophores	sis (Chamber) Buffer
EDVOTEK Model #	Concentrated Buffer (50x)	Distilled + Water	Total = Volume
M6	4 ml	196 ml	200 ml
M6 +	6 ml	294 ml	300 ml
M12, M20	8 ml	392 ml	400 ml
M36	10 ml	490 ml	500 ml

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Agarose Gel Preparation, continued

Useful Hint

To facilitate the delivery of

of the bulb.

small sample volumes

with transfer

pipets, gently

pipet stem, instead

squeeze the

If using transfer

pipets for sample

delivery, load the sample well until it is full.

- 15. Make sure the gel is completely covered with buffer. The agarose gel is sometimes called a "submarine gel" because it is submerged under buffer for sample loading and electrophoretic separation.
- 16. Load samples in wells and conduct electrophoresis according to experiment instructions starting on page 15.

Sample Delivery and Practice Gel Loading

An automatic micropipet is used to deliver accurate, reproducible volumes of sample. For gels to be stained with Methylene Blue Plus[™] or DNA Blue InstaStain[™], load the sample well with 40 microliters of sample. Check with your instructor regarding the amount of sample you should be delivering.

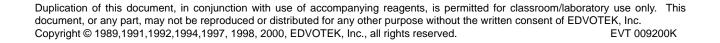
With the EDVOTEK system, an alternative sample delivery method with disposable microtipped transfer pipets can be used. Transfer pipets are not precise, and because their volumes can not be accurately controlled, significant sample waste can occur. Delivery of small sample volumes with transfer pipets can be facilitated by gently squeezing the pipet stem, instead of the bulb.

PRACTICE GEL LOADING

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



- 1. Cast a gel with the maximum number of wells and 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 under water.
- 2. Practice delivering the practice solution to the sample wells. Take care not to damage or puncture the wells with the pipet tip.
- 3. If you need more practice, remove the practice gel loading solution by squirting buffer into the wells with a transfer pipet.
- 4. Replace the practice gel with a fresh gel for the actual experiment. The practice gel loading solution is diluted in the buffer and will not interfere with the experiment.

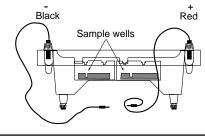


EXPERIMENTAL PROCEDURES

Conducting Agarose Gel Electrophoresis

Reminder:

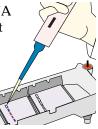
During electrophoresis, the DNA samples migrate through the agarose gel towards the positive electrode. Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.



Have a waterbath or beaker of water warmed to 65°C for heating the tubes containing DNA fragments before gel loading. At 65°C, non-specific aggregation due to sticky ends generated by restriction enzyme digestions will melt. This will result in sharp individual DNA bands upon separation by agarose gel electrophoresis.

LOADING DNA SAMPLES

- 1. Heat the samples, including the Standard DNA fragments (Component E) for two minutes at 65°C. Allow the samples to cool for a few minutes.
- 2. Load the first well of each row of samples with 40 μl of the Standard DNA fragments (E).



3. Load 40 µl of the reaction and control samples into separate wells.

If samples from various groups are being electrophoresed on a shared gel, be sure to note the sample wells in which each group's control and reaction samples are loaded.

RUNNING THE GEL

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

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

- 2. 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).
- 3. Set the power source at the required voltage and run the electrophoresis for the length of time as determined by your instructor. General guidelines are presented in Table C.
- 4. Check to see that current is flowing properly you should see bubbles forming on the electrodes.

Table C: Time and Voltage		
Volts*	Recomme	nded Time
Voits	Minimum	Optimal
50	60 min	2.0 hrs
70	40 min	1.5 hrs
125	30 min	45 min

* The EDVOTEK Model #M6 should not be run at higher than 70 volts.

•

EDVOTEK • The Biotechnology Education Company ®	EXPERIMENTAL PROCEDURES
	5. Allow the tracking dye to migrate 3.5 to 4 centimeters from the wells for adequate separation of the DNA bands.

- 6. After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads and remove the cover.
- 7. Remove the gel on its bed. Place your hands on each end of the gel to prevent it from slipping off the bed.
- 8. Transfer the gel from the bed to stain the gel for DNA Visualization with DNA Blue InstaStain[™] or Methylene Blue Plus[™].

EXPERIMENTAL PROCEDURES

Staining & Visualization of DNA





WEAR SAFETY GOGGLES AND GLOVES

Advantages of DNA Blue InstaStain™ vs. Liquid Staining

- Safe and Simple to Use
- Quick 15-minute staining
- Uniformity of Staining
- Minimal liquid waste

DNA BLUE INSTASTAINTM

Many EDVOTEK electrophoresis experiments now feature **InstaStain[™]**, a new proprietary staining method for staining DNA separated on agarose gels. Based on state-of-the-art technology, this experiment includes DNA Blue InstaStain[™], which is a safe, quick, non-liquid method for staining gels. It minimizes the mess of conventional DNA staining with blue stains.

Staining with DNA Blue InstaStain[™]

- 1. After electrophoresis is completed, place the gel on a flat surface. Moisten the gel with several drops of electrophoresis buffer.
- 2. Wearing gloves, place the blue side of the DNA Blue InstaStain sheet on the well-moistened gel.
- 3. Firmly run your fingers over the entire surface of the DNA InstaStain.

Do this several times.

4. Place the gel and DNA Blue InstaStain on a piece of plastic wrap. Then put the gel casting tray and a small empty beaker on top.

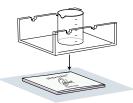
This will ensure that the InstaStain sheet maintains good contact with the gel surface.

Allow the DNA Blue InstaStainTM to sit for 15 minutes.









DNA Blue InstaStain[™](Cat. #2003) contains Methylene Blue. Also available is DNA InstaStain[™](Cat. #2001), which contains ethidium bromide.

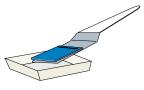
Staining & Visualization of DNA, cont.

Useful Hint!
A large weigh boat works well for destaining a 7 x 7 cm gel. Use 50 ml of
37°C distilled water to submerge the gel in a large weigh boat.

EXPERIMENTAL PROCEDURES

Destaining and Visualization of DNA

5. After 15 minutes, remove the sheet of DNA Blue InstaStain and transfer the gel to a large weigh boat or small plastic container.



- 6. Conduct destaining with distilled water that has been warmed to 37° C.
 - First destain: submerge the gel under a small amount of 37°C distilled water for 10 minutes with occasional agitation.
 - Second and third destain: submerge the gel under a small amount of 37°C distilled water for another 10 minutes with occasional agitation.
- 7. After the first destain, the larger DNA bands will be visible as dark blue bands against a lighter blue background. When com-

pletely destained, the dark blue DNA bands will become clearer and the entire background will become uniformly light blue in color.

- 8. Carefully remove the gel from the destain solution and examine the gel on a Visible Light Gel Visualization System. To optimize visibility, use the amber filter provided with EDVOTEK equipment.
- 9. If the gel is too light and bands are difficult to see, repeat the staining and destaining procedures.

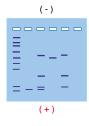
Storage and Disposal of Gel

• A gel stained with DNA Blue InstaStain[™] may be stored in the refrigerator for several weeks. Place the gel in a sealable plastic bag with destaining liquid.

DO NOT FREEZE AGAROSE GELS.

• Stained gels which are not kept can be discarded in solid waste disposal.

DO NOT EXCEED 37°C ! Warmer temperatures will soften the gel and may cause it to break.

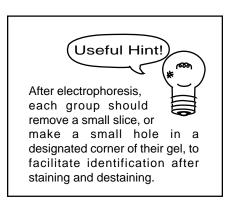


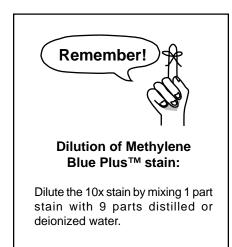
EXPERIMENTAL PROCEDURES

Staining & Visualization of DNA, cont.



WEAR SAFETY GOGGLES AND GLOVES





TRADITIONAL LIQUID STAINING WITH METHYLENE BLUE PLUSTM

1. Remove each gel from its bed and totally submerge the gel(s) in one tray containing 600 ml of diluted Methylene Blue Plus[™] stain.

Do not stain gel(s) in the electrophoresis apparatus.

- 2. Stain gel(s) for a minimum of 30 minutes, with occasional agitation.
- 3. Conduct destaining in 600 ml of distilled water that has been warmed to 37° C.
 - First destain: completely submerge the gel(s) in 600 ml of 37°C distilled water for 15 minutes with occasional agitation. Then discard the destaining solution
 - Second and third destain: completely submerge the gel(s) in 600 ml of 37°C distilled water for another 15 minutes with occasional agitation.

DO NOT EXCEED 37°C ! Warmer temperatures will soften the gel and may cause it to break.

Bands will start to become clearly visible after the second destain. You may also leave the gel(s) in destain overnight.

- 5. Carefully remove the gel from the destain solution and examine on a Visible Light Gel Visualization System. To optimize visibility, use the amber filter provided with EDVOTEK equipment.
- 6. If the gel is too light and bands are difficult to see, repeat the staining and destaining procedures.

Storage and Disposal of Gel

• Gels stained with Methylene Blue Plus[™] may be stored in the refrigerator for several weeks. Place the gel in a sealable plastic bag with destaining liquid.

DO NOT FREEZE AGAROSE GELS.

• Stained gels which are not kept can be discarded in solid waste disposal.

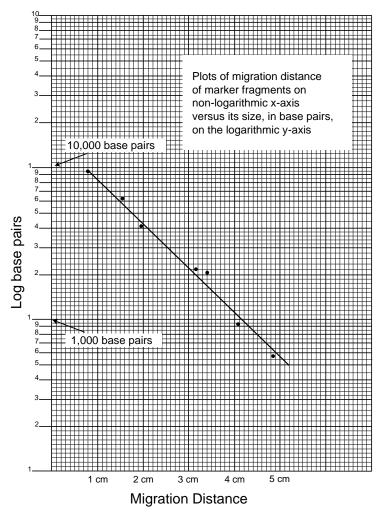
EXPERIMENTAL PROCEDURES

Size Determination of DNA Fragments

The sizes of the fragments obtained from the *Eco* RI digest of lambda DNA will be extrapolated by their migration distances relative to the Standard DNA Fragments (E), for which the size of each fragment is known. The assignment of sizes for DNA fragments separated by agarose gel electrophoresis can have $\pm 10\%$ margin of error.

1. Measure and record the distance traveled in the agarose gel by each Standard DNA fragment (except the largest 23,130 bp fragment, which will not fit in a straight line in step 4).

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).

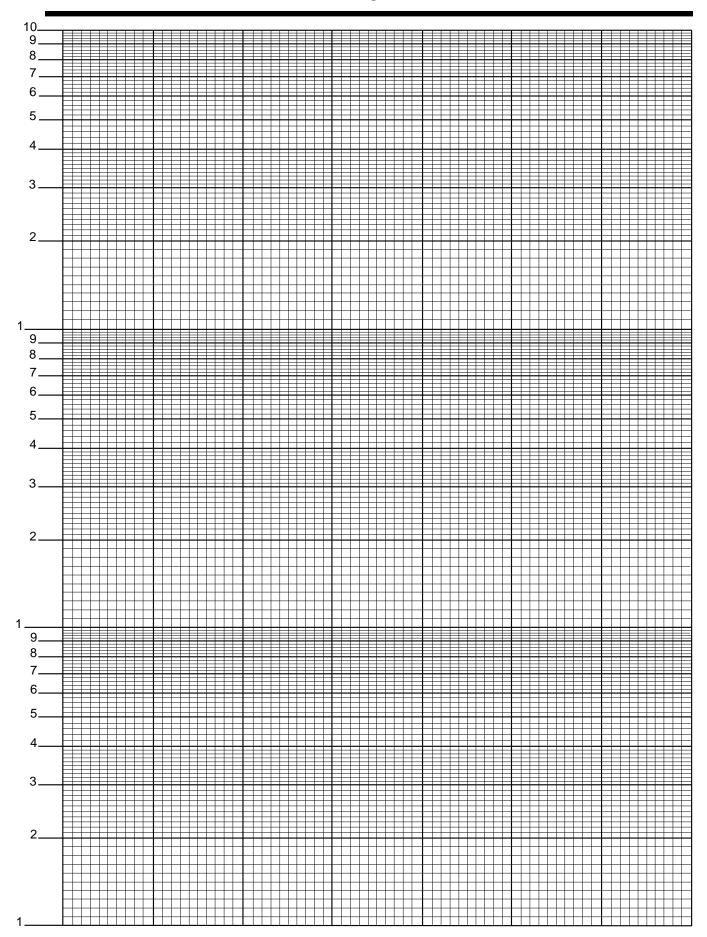


- 2. Label the semi-log graph paper:
 - A. Label the non-logarithmic horizontal x-axis "Migration Distance" in centimeters at equal intervals.
 - B. Label the logarithmic vertical y-axis "Log base pairs". Choose your scales so that the data points are well spread out. Assume the first cycle on the yaxis represents 100-1,000 base pairs and the second cycle represents 1,000-10,000 base pairs.
- 3. For each Standard DNA fragment, 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 Figure 3 for an example).

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Figure 3



EDVO-Kit # 212: Cleavage of Lambda DNA with Eco RI Endonuclease 21

					Section V - Reactivity	Data					
		aterial Safety Data SI to comply with OSHA's Hazard		1	Stability	Unstable			ins to Avoid		
(edvotek "		CFR 1910.1200 Standard mus specific requirements.			1	Stable	X		None		
					Hazardous Decomposition or B	available					
IDENTITY (As Used on Label and List) Agarose		Note: Blank spaces are applicable, or no informa be marked to indicate th	ation is available.	the space must		1 1		C ditio			
Section I					Hazardous Polymerization	May Occur	x	Conditio	ons to Avoid		
Manufacturer's Name		Emergency Telephone Nur	mber (201) 2	51-5000	Section VI - Health H		X		Non	ie	
EDVOTEK, Inc.		Talaahaa Numbaa (as is (assa		51-5990	Route(s) of Entry:	Inhalation	?		Skin? v		Ingestion? Yes
Address (Number, Street, City, State,	Zip Code)	Telephone Number for inform		51-5990			Yes		Y Y	'es	Yes
14676 Rothgeb Drive		Date Prepared 8/25/97			Health Hazards (Acute and C Inhalation:	No data available		Ingestic	on: Large a	mounts may	cause diarrhea
Rockville, MD 20850		Signature of Preparer (option	al)		Carcinogenicity:	NTP?			C Monograp		OSHA Regulation?
		3 (-F	,		Signs and Symptoms of Exp	osure No data a	vailable				
Section II - Hazardous Ingred	lients/Iden				Medical Conditions General						
Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA	PEL ACGIH TLV Red	ther Limits commended	% (Optional)				• No o	data availab	ole	
This product contains no hazardous n	naterials as de	fined by the OSHA Hazard	Communicatio	n	Emergency First Aid Proced	lures Treat sym	ptomatio	cally an	d supportive	ely	
Standard. CAS #9012-36-6											
Section III - Physical/Chemic	al Charact	eristics			Section VII - Precauti				nd Use		
	194° F	Specific Gravity (H ₂ 0 = 1)		No data	Steps to be Taken in case M	laterial is Release Sweep up and pl			container fo	or disposal	
Boiling Point For 1% solution	194° F	Specific Gravity (F20 = 1)		No data		Sweep up and pi	ace in si		container 10	n uisposai	
Vapor Pressure (mm Hg.)	No data	Melting Point Evaporation Rate		No data	Waste Disposal Method	Normal solid wa	ste disp	osal			
Vapor Density (AIR = 1)	No data	(Butyl Acetate = 1)		No data	Precautions to be Taken in H	andling and Stori	ng				
Solubility in Water Insoluble - cold						None					
Appearance and Odor White p	owder, no odo	r			Other Precautions						
			4.0			None					
Section IV - Physical/Chemic Flash Point (Method Used)		Flammable Limits	LEI	UEL	Section VIII - Control						
No data			N.D.	N.D.	Respiratory Protection (Spe	cify Type) Cher	nical car	rtridge r	espirator wi	ith full facep	iece.
Extinguishing Media Water spray, dr	y chemical, ca	rbon dioxide, halon or stand	ard foam		Ventilation	Local Exhaust				Special	
Special Fire Fighting Procedures					Protective Claves	Mechanical (Gene	eral)Gen				
Possible fire ha	zard when exp	osed to heat or flame			Yes				Eye Protect	stion Splas	sh proof goggles
Unusual Fire and Explosion Hazards	None				Other Protective Clothing or	Equipment Im	pervious	s clothir	ng to preven	nt skin contac	rt -
					Work/Hygienic Practices	No	ne				
-					Castian V. Desetivity	Data					
	N	laterial Safety Data S	iheet		Section V - Reactivity			Conditio	ons to Avoid		
California ®	May be used	laterial Safety Data S	d Communicatio		Stability	Unstable	X		ons to Avoid None		
(edvotek®	May be used		d Communicatio		Stability	Unstable	Х				
	May be used Standard. 29	I to comply with OSHA's Hazar CFR 1910.1200 Standard mu specific requirements.	d Communications be consulted	for	Stability	Unstable Stable Strong oxidizir	X ng agent	ts	None	ide	
IDENTITY (As Used on Label and List) 50x Electrophoresis	May be used Standard. 29	to comply with OSHA's Hazar CFR 1910.1200 Standard mu	d Communication ist be consulted e not permitted. It nation is available	for	Stability Incompatibility Hazardous Decomposition or B	Unstable Stable Strong oxidizir iyproducts Carb	X ng agent	ts oxide, C	None Carbon dioxi		
	May be used Standard. 29	I to comply with OSHA's Hazar CFR 1910.1200 Standard mu specific requirements. Note: Blank spaces ar applicable, or no inform	d Communication ist be consulted e not permitted. It nation is available	for	Stability	Unstable Stable Strong oxidizir syproducts May Occur	X ng agent	ts oxide, C	None Carbon dioxi ons to Avoid		
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50x Electrophoresis	May be used Standard. 29	Ito comply with OSHA's Hazar CFR 1910.1200 Standard mu specific requirements. Note: Blank spaces an applicable, spaces on inform be marked to indicate t Emergency Telephone Nu	d Communication ist be consulted e not permitted. I nation is available hat. (301) 2	for	Stability Incompatibility Hazardous Decomposition or B Hazardous Polymerization	Unstable Stable Strong oxidizir yproducts Carb May Occur Will Not Occur	X ng agent oon mone X	ts oxide, C	None Carbon dioxi ons to Avoid None		Ingestion? Yes
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50x Electrophoresis Section I Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, 14676 Rothgeb Drive Rockville, MD 20850 Section II - Hazardous Ingree	May be usec Standard. 29 s Buffer . Zip Code) dients/Iden	It to comply with OSHA's Hazar CFR 1910.1200 Standard mu specific requirements. Note: Blank spaces an applicable, or no inform be marked to indicate t Emergency Telephone Nu Telephone Number for inform Date Prepared Signature of Preparer (option tify Information	d Communicatic ist be consulted e not permitted. I nation is available hat. (301) 2 (301) 2 (99/15/9 nal)	for any item is not the space must 251-5990 251-5990	Stability Incompatibility Hazardous Decomposition or B Hazardous Polymerization Section VI - Health H Route(s) of Entry: Health Hazards (Acute and C Carcinogenicity: None ident	Unstable Stable Strong oxidizir yproducts May Occur Will Not Occur azard Data Inhalation Chronic) None tified NTP? posure Irritation	X ng agent on mone X ? Yes to uppe	ts oxide, C Condition IAR(er respira	None Carbon dioxi ons to Avoid None Skin? Y C Monograp atory tract, s	Yes phs?	
50x Electrophoresis Section I Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, 14676 Rothgeb Drive Rockville, MD 20850 Section II - Hazardous Ingreet Hazardous Components [Specific Chemical Identity; Common Name(s)]	May be usec Standard. 29 s Buffer Zip Code) dients/Iden	Ito comply with OSHA's Hazar CFR 1910.1200 Standard mu specific requirements. Note: Blank spaces an applicable, or no inform be marked to indicate t Emergency Telephone Nu Telephone Number for inform Date Prepared Signature of Preparer (option tify Information	d Communicatic ist be consulted e not permitted. I hation is available hat. (301) 2 (301) 2 (9/15/9 nal) Dther Limits acommended	for any item is not the space must 251-5990 251-5990	Stability Incompatibility Hazardous Decomposition or B Hazardous Polymerization Section VI - Health H Route(s) of Entry: Health Hazards (Acute and C Carcinogenicity: None ident Signs and Symptoms of Exp Medical Conditions General	Unstable Stable Strong oxidizir yproducts Carb May Occur Will Not Occur azard Data Inhalation Chronic) None tified NTP? posure Irritation ly Aggravated by I	X Ig agent ion mone X ? Yes to uppe Exposure	ts oxide, C Condition IAR(er respira re <u>N</u>	None Carbon dioxi ons to Avoid None Skin? Y C Monograp atory tract, s Vone	Yes phs? skin, eyes	OSHA Regulation?
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50x Electrophoresis Section I Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, 14676 Rothgeb Drive Rockville, MD 20850 Section II - Hazardous Ingreet Hazardous Components [Specific Chemical Identity; Common Name(s)] This product contains no hazard Communication Standard.	May be used Standard. 29 s Buffer . Zip Code) dients/Iden 	Ito comply with OSHA's Hazar CFR 1910.1200 Standard mu specific requirements. Note: Blank spaces an applicable, or no inform be marked to indicate t Emergency Telephone Nu Telephone Number for inform Date Prepared Signature of Preparer (option tify Information APEL ACGIH TLV Re as defined by the OSHA Haz	d Communicatic ist be consulted nation is available hat. (301) 2 (301) 2 (9/15/9 nai) Dther Limits acommended zard	for any item is not the space must 251-5990 251-5990 7	Stability Incompatibility Hazardous Decomposition or B Hazardous Polymerization Section VI - Health H Route(s) of Entry: Health Hazards (Acute and G Carcinogenicity: None ident Signs and Symptoms of Exp Medical Conditions General Emergency First Aid Procec Eyes: Flush with water Section VII - Precauti Steps to be Taken in case M	Unstable Stable Strong oxidizir yproducts Carb May Occur Will Not Occur azard Data Inhalation Chronic) None tified NTP? posure Irritation ly Aggravated by I dures Ingestion Inhalation: Mo ons for Safe laterial is Release	x in g agentition in a second	ts oxide, C Conditie IAR(er respira re <u>n</u> scious, p scious, p air esh air ing ar	None Carbon dioxi ons to Avoid None Skin? Y C Monograp atory tract, s Jone give large a Skin du Use	Yes phs? skin, eyes mounts of war v Wash with e protective of	OSHA Regulation? ater soap and water
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50x Electrophoresis Section I Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, 14676 Rothgeb Drive Rockville, MD 20850 Section II - Hazardous Ingreet Hazardous Components [Specific Chemical Identity: Common Name(s)] This product contains no hazard Communication Standard. Section III - Physical/Chemic Boiling Point Vapor Pressure (mm Hg.) Vapor Density (AIR = 1) Setubility in Mater	May be used Standard. 29 s Buffer Zip Code) dients/Iden OSH/ ous materials No data No data No data	Ito comply with OSHA's Hazar CFR 1910.1200 Standard mu specific requirements. Note: Blank spaces an applicable, or no inforr be marked to indicate t Emergency Telephone Nu Telephone Number for inforr Date Prepared Signature of Preparer (optior tify Information NPEL ACGIH TLV Re as defined by the OSHA Haz specific Gravity (H ₂ 0 = 1 Melting Point Evaporation Rate (Butyl Acetate = 1)	d Communicatic ist be consulted nation is available hat. (301) 2 (301) 2 (9/15/9 nai) Dther Limits acommended zard	for if any item is not the space must 251-5990 251-5990 7 % (Optional) No data No data	Stability Incompatibility Hazardous Decomposition or B Hazardous Decomposition or B Hazardous Polymerization Section VI - Health H Route(s) of Entry: Health Hazards (Acute and t Carcinogenicity: None ident Signs and Symptoms of Exp Medical Conditions General Emergency First Aid Procee Eyes: Flush with water Section VII - Precauti Steps to be Taken in case M and rinse wit Waste Disposal Method Precautions to be Taken in F	Unstable Stable Strong oxidizir yproducts Carb May Occur Will Not Occur azard Data Inhalation Chronic) None tified NTP? vosure Irritation Iy Aggravated by I tures Ingestion Inhalation: Mo cons for Safe Interial is Release th water, or collect Dispose in accord: enviromental regu	x g agent on mon x ? Yes to uppe Exposur : If conus i in abso ance wit lations.	ts oxide, C Conditii respira respira respira eshair ing ar illed W rptive n	None Carbon dioxi ons to Avoid None Skin? Y atory tract, s None give large a Skin dUSE Vear suitable	Yes phs? skin, eyes mounts of w. :: Wash with e protective e dispose of th	OSHA Regulation? ater a soap and water clothing. Mop up spill te absorptive material.
50x Electrophoresis Section I Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, 14676 Rothgeb Drive Rockville, MD 20850 Section II - Hazardous Ingreet Hazardous Components [Specific Chemical Identity: Common Name(s)] This product contains no hazard Communication Standard. Section III - Physical/Chemic Boiling Point Vapor Pressure (mm Hg.) Vapor Density (AIR = 1) Setubility in Mater	May be usec Standard. 29 s Buffer Zip Code) dients/Iden OSH4 ous materials : al Charact No data No data No data (greater than 1	Ito comply with OSHA's Hazar CFR 1910.1200 Standard mu specific requirements. Note: Blank spaces an applicable, or no inforr be marked to indicate t Emergency Telephone Nu Telephone Number for inforr Date Prepared Signature of Preparer (option tify Information NPEL ACGIH TLV Re as defined by the OSHA Haz Eristics Specific Gravity (H_0 = 1 Melting Point Evaporation Rate (Butyl Acetate = 1) 0%)	d Communicatic ist be consulted nation is available hat. (301) 2 (301) 2 (9/15/9 nai) Dther Limits acommended zard	for if any item is not the space must 251-5990 251-5990 7 % (Optional) No data No data	Stability Incompatibility Hazardous Decomposition or B Hazardous Decomposition or B Hazardous Polymerization Section VI - Health H Route(s) of Entry: Health Hazards (Acute and t Carcinogenicity: None ident Signs and Symptoms of Exp Medical Conditions General Emergency First Aid Procee Eyes: Flush with water Section VII - Precauti Steps to be Taken in case M and rinse wit Waste Disposal Method Precautions to be Taken in F	Unstable Stable Strong oxidizir yproducts May Occur Will Not Occur azard Data Inhalation Chronic) None tified NTP? ified NTP? ified NTP? ified NTP? ified NTP? ified Inhalation: Mone ified NTP? ified N	x g agent on mon x ? Yes to uppe Exposur : If conus i in abso ance wit lations.	ts oxide, C Conditii respira respira respira eshair ing ar illed W rptive n	None Carbon dioxi ons to Avoid None Skin? Y atory tract, s None give large a Skin dUSE Vear suitable	Yes phs? skin, eyes mounts of w. :: Wash with e protective e dispose of th	OSHA Regulation? ater a soap and water clothing. Mop up spill te absorptive material.
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Sox Electrophoresis Section I Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, 14676 Rothgeb Drive Rockville, MD 20850 Section II - Hazardous Ingree Hazardous Components [Specific Chemical Identity, Common Name(s)] This product contains no hazard Communication Standard. Section III - Physical/Chemic Boiling Point Vapor Pressure (mm Hg.) Vapor Density (AIR = 1) Solubility in Water Appreciable, 4 Appearance and Odor Clear, liquid, Section IV - Physical/Chemic	May be usec Standard. 29 s Buffer Zip Code) dients/Iden OSH/ ous materials al Charact No data No data No data (greater than 1 slight vinegar	to comply with OSHA's Hazar CFR 1910.1200 Standard mu specific requirements. Note: Blank spaces an applicable, or no inform be marked to indicate t Emergency Telephone Nu Telephone Number for inform Date Prepared Signature of Preparer (option tify Information APEL ACGIH TLV Re as defined by the OSHA Haz specific Gravity (H20 = 1 Melting Point Evaporation Rate (Butyl Acetate = 1) 0%) odor teristics N.D. = N	d Communicatic ist be consulted e not permitted. I hation is available hat. (301) 2 (301) 2 (301) 2 (09/15/9 nail) Dther Limits ecommended card	for the space must th	Stability Incompatibility Hazardous Decomposition or B Hazardous Decomposition or B Hazardous Polymerization Section VI - Health H Route(s) of Entry: Health Hazards (Acute and t Carcinogenicity: None ident Signs and Symptoms of Exp Medical Conditions General Emergency First Aid Proceet Eyes: Flush with water Section VII - Precautit Steps to be Taken in case M and rinse wit Waste Disposal Method Precautions to be Taken in H Other Precautions Section VIII - Control	Unstable Strong oxidizir yproducts Carb May Occur Will Not Occur azard Data Inhalation Chronic) None iffied NTP? posure Irritation ly Aggravated by I dures Ingestion Inhalation: Mo inhalation:	x g agent on mon x ? Yes to uppe Exposur : If conus i in abso ance wit lations.	ts oxide, C Conditii respira respira respira eshair ing ar illed W rptive n	None Carbon dioxi ons to Avoid None Skin? Y atory tract, s None give large a Skin dUSE Vear suitable	Yes phs? skin, eyes mounts of w. :: Wash with e protective e dispose of th	OSHA Regulation? ater a soap and water clothing. Mop up spill te absorptive material.
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Source of the second seco	May be usec Standard. 29 s Buffer Zip Code) dients/iden OSH/ ous materials : al Charact No data No data No data No data (greater than 1 slight vinegar cal Characc	to comply with OSHA's Hazar CFR 1910.1200 Standard mu specific requirements. Note: Blank spaces an applicable, or no inform be marked to indicate t Emergency Telephone Nu Telephone Number for inform Date Prepared Signature of Preparer (option tify Information APEL ACGIH TLV Re as defined by the OSHA Haz specific Gravity (H20 = 1 Melting Point Evaporation Rate (Butyl Acetate = 1) 0%) odor teristics N.D. = N	d Communicatic ist be consulted e not permitted. I hation is available hat. (301) 2 (301) 2 (301) 2 (301) 2 (09/15/9 nail) Dther Limits ecommended card	for if any item is not the space must 251-5990 251-5990 7 % (Optional) No data No data No data UEL N.D.	Stability Incompatibility Hazardous Decomposition or B Hazardous Decomposition or B Hazardous Polymerization Section VI - Health H Route(s) of Entry: Health Hazards (Acute and t Carcinogenicity: None ident Signs and Symptoms of Exp Medical Conditions General Emergency First Aid Proceet Eyes: Flush with water Section VII - Precautit Steps to be Taken in case M and rinse wit Waste Disposal Method Precautions to be Taken in H Other Precautions Section VIII - Control	Unstable Stable Strong oxidizir yproducts Carb May Occur Will Not Occur azard Data Inhalation Chronic) None iffied NTP? vosure Irritation Iy Aggravated by I dures Ingestion Inhalation: Mo ons for Safe Iaterial is Release th water, or collect Dispose in accord: enviromental regu -landling and Stori Avoid eye and ski None Measures cify Type) Local Exhaust	x	ts Oxide, C Condition IAR(r respiration r respiration scious, p scious, scious, p scious, scious, s	None Carbon dioxi ons to Avoid None Skin? Y C Monograp atory tract, s Vone give large au Skin nd Use Vear suitable naterial and plicable fede	Yes phs? skin, eyes mounts of w. :: Wash with e protective e dispose of th leral, state, ar leral, state, ar	OSHA Regulation? ater asoap and water clothing. Mop up spill ae absorptive material. ad local None
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					Section V - Reactivit	y Data					
	N	laterial Safety Data S	heet		Stability	Unstable		Conditions to Avoid			
edvotek [®]	Standard. 29	to comply with OSHA's Hazard CFR 1910.1200 Standard must specific requirements.	st be consulted f	n for	Stable X None						
					Hazardous Decomposition or Byproducts						
IDENTITY (As Used on Label and List) Methylene Blue I	Plus ™	applicable, or no inform	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.			nonoxide, Carbon	dioxide,		lfur oxides	, hydrogen, chloride gas	
Section I		be marked to indicate that.			Hazardous Polymerization	May Occur		Conditions to Avoid			
Manufacturer's Name		Emergency Telephone Nu	mber (201) 2	51-5000	Section VI - Health I	Will Not Occur	Х	None			
EDVOTEK, Inc.		Telephone Number for inform	(301) 251-5990			Inhalatio	n?	Skin?	-	Ingestion? Yes	
Address (Number, Street, City, State,	Zip Code)			51-5990	Health Hazards (Acute and		Yes	Y	'es		
14676 Rothgeb Drive	Date Prepared 8/25/97			Skin: May cause ski		lyes: Ma	y cause eye irritatio		halation: Cyanosis		
Rockville, MD 20850		Signature of Preparer (option	nal)		Carcinogenicity: NTP? IARC Monographs? OSHA Regul Meets criteria for proposed OSHA medical records rule PEREAC 47.30420.82						
					Signs and Symptoms of Ex	posure No da	ta availal	ole			
Section II - Hazardous Ingred	lients/lder		ther Limits		Medical Conditions Genera	ally Aggravated by	Exposu	e No data availa	ble		
Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSH/	A PEL ACGIH TLV Re	commended	% (Optional)	Emergency First Aid Proce	dures					
Methylene Blue	3.7 Bis (Dimethylamino) Phenothiazin 5 IUM Chloride No data available					Treat syr	nptomati	cally			
CAS # 61-73-4	5 IUM Chie	nde no data available			0 (1) /// D						
Section III - Physical/Chemic	al Charact	teristics			Section VII - Precaut Steps to be Taken in case						
Boiling Point	Boiling Point No data Spe			No data	Steps to be Taken in case			l wash spill site			
Vapor Pressure (mm Hg.)	No data	Melting Point		No data	Waste Disposal Method	Mix mat	erial with	a combustible solv	ent and bu	rn in chemical	
Vapor Density (AIR = 1)	No data	Evaporation Rate		No data	incinerator equipped wit	th afterburner and	scrubber	. Check local and s	tate regula	tions.	
Solubility in Water Soluble - cold		(Butyl Acetate = 1)		110 uuu	Precautions to be Taken in Keep tightly closed. Sto	-	-				
Appearance and Odor Blue solution,					Other Precautions	, ary pa					
					None						
Section IV - Physical/Chemic	al Charac		LEL	UEL	Section VIII - Contro	l Measures					
Flash Point (Method Used) No data ava	ulable	Flammable Limits	No data	No data	Respiratory Protection (Sp	ecify Type) MI	OSH/OS	HA approved, SCB	A		
Extinguishing Media					Ventilation	Local Exhaust			Special		
Water spray Special Fire Fighting Procedures	, carbon diox	ide, dry chemical powder, al	cohol or polym	her foam		Mechanical (Ge	neral)	Required	Other		
Self contained breathing app	aratus and pr	otective clothing to prevent c	ontact with ski	n and eyes	Protective Gloves Rubb	er		Eye Protec	tion Ch	em. safety goggles	
					Other Protective Clothing o	r Equipment	Rubber l	poots			
Unusual Fire and Explosion Hazards	Emits toxid	fumes under fire conditions			Work/Hygienic Practices						
					Section V - Reactivit	ty Data					
	N	laterial Safety Data S	heet		Stability	Unstable	1	Conditions to Avoid			
Cedvotek®	May be used	to comply with OSHA's Hazard CFR 1910.1200 Standard mu	d Communication		Clubinty	Stable	X	None			
Certoich	Stanuaru. 25	specific requirements.	si be consulted i	01	Incompatibility	None known					
IDENTITY (As Used on Label and List)		Note: Blank spaces are	e not permitted. If	any item is not	Hazardous Decomposition or	Byproducts					
Gel loading solution con	ncentrate, 1	applicable or no inform	ation is available, hat.	the space must	Hazardous	Sulfur oxides		Conditions to Avoid	1		
Section I					Polymerization	Will Not Occur	X	None	-		
Manufacturer's Name			mber								
		Emergency Telephone Nu	(301) 2	251-5990	Section VI - Health	Hazard Data					
EDVOTEK, Inc.	T 0 1)	Telephone Number for inform	(301) 2 nation		Section VI - Health Route(s) of Entry:	Inhalatio	on?	Skin?		Ingestion?	
EDVOTEK, Inc. Address (Number, Street, City, State,	Zip Code)	Telephone Number for inform	(301) 2 nation (301) 2	251-5990 251-5990	Route(s) of Entry: Health Hazards (Acute and	Inhalatio Yes d Chronic)		Yes		Ingestion? Yes	
Address (Number, Street, City, State, 14676 Rothgeb Drive	Zip Code)		(301) 2 nation (301) 2		Route(s) of Entry: Health Hazards (Acute and Acute eye contact: May	Inhalatio Yes d Chronic) y cause irritation		Yes ta available for othe		Yes	
Address (Number, Street, City, State,	Zip Code)	Telephone Number for inform	(301) 2 nation (301) 2 17		Route(s) of Entry: Health Hazards (Acute and	Inhalatio Yes d Chronic) y cause irritation NTP?		Yes		Ingestion? Yes OSHA Regulation? No data	
Address (Number, Street, City, State, 14676 Rothgeb Drive Rockville, MD 20850		Telephone Number for inform Date Prepared 12/17/9 Signature of Preparer (option	(301) 2 nation (301) 2 17		Route(s) of Entry: Health Hazards (Acute and Acute eye contact: May Carcinogenicity: None Signs and Symptoms of E:	Inhalatic Yes d Chronic) y cause irritation NTP? No xposure	No da	Yes ta available for othe IARC Monogra		Yes OSHA Regulation?	
Address (Number, Street, City, State, 14676 Rothgeb Drive Rockville, MD 20850 Section II - Hazardous Ingred		Telephone Number for inform Date Prepared 12/17/9 Signature of Preparer (option titify Information	(301) 2 nation (301) 2 17 nal)		Route(s) of Entry: Health Hazards (Acute and Acute eye contact: May Carcinogenicity: None Signs and Symptoms of E: May cause skin or eye Medical Conditions Gener	Inhalatio Yes d Chronic) y cause irritation NTP? No xposure irritation	No da	Yes ta available for othe IARC Monogra No data		Yes OSHA Regulation?	
Address (Number, Street, City, State, 14676 Rothgeb Drive Rockville, MD 20850 Section II - Hazardous Ingred Hazardous Components [Specific Chemical Identity: Common Name(s)]	lients/Ider	Telephone Number for inform Date Prepared 12/17/9 Signature of Preparer (option tify Information APEL ACGIH TLV Re	(301) 2 hation (301) 2 17 hal) http://www.commended		Route(s) of Entry: Health Hazards (Acute and Acute eye contact: May Carcinogenicity: None Signs and Symptoms of E: May cause skin or eye Medical Conditions Gener None reported	Inhalatic Yes d Chronic) y cause irritation NTP? No xposure irritation ally Aggravated b	No da	Yes ta available for othe IARC Monogra No data		Yes OSHA Regulation?	
Address (Number, Street, City, State, 14676 Rothgeb Drive Rockville, MD 20850 Section II - Hazardous Ingred Hazardous Components [Specific Chemical Identity: Common Name(s)] This product contains no hazardo	lients/Ider	Telephone Number for inform Date Prepared 12/17/9 Signature of Preparer (option tify Information APEL ACGIH TLV Re	(301) 2 hation (301) 2 7 hal) http://www.commended	51-5990	Route(s) of Entry: Health Hazards (Acute and Acute eye contact: May Carcinogenicity: None Signs and Symptoms of E: May cause skin or eye Medical Conditions Gener None reported Emergency First Aid Proc Treat syn	Inhalatic Yes d Chronic) y cause irritation NTP? No xposure irritation ally Aggravated b edures nptomatically and	No da data y Exposu supporti	Yes tta available for othu IARC Monogra No data re		Yes OSHA Regulation?	
Address (Number, Street, City, State, 14676 Rothgeb Drive Rockville, MD 20850 Section II - Hazardous Ingred Hazardous Components [Specific Chemical Identity: Common Name(s)]	lients/Ider	Telephone Number for inform Date Prepared 12/17/9 Signature of Preparer (option tify Information APEL ACGIH TLV Re	(301) 2 hation (301) 2 7 hal) http://www.commended	51-5990	Route(s) of Entry: Health Hazards (Acute and Acute eye contact: May Carcinogenicity: None Signs and Symptoms of E: May cause skin or eye Medical Conditions Gener None reported Emergency First Aid Proc Treat syn Rinse con	Inhalatic Yes d Chronic) y cause irritation NTP? No xposure irritation ally Aggravated b edures nptomatically and ntacted area with o	No da data y Exposu supporti copious a	Yes tta available for othe IARC Monogra No data re vely mounts of water.		Yes OSHA Regulation?	
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Address (Number, Street, City, State, 14676 Rothgeb Drive Rockville, MD 20850 Section II - Hazardous Ingred Hazardous Components [Specific Chemical Identity: Common Name(s)] This product contains no hazardo Communication Standard.	l ients/Ider OSH/ Dus materia	Telephone Number for inform Date Prepared 12/17/9 Signature of Preparer (option tify Information A PEL ACGIH TLV Re Is as defined by the OSH	(301) 2 nation (301) 2 7 ial) Pther Limits ccommended A Hazard	51-5990	Route(s) of Entry: Health Hazards (Acute and Acute eye contact: May Carcinogenicity: None Signs and Symptoms of E: May cause skin or eye Medical Conditions Gener None reported Emergency First Aid Proc Treat syn Rinse con	Inhalatic Yes d Chronic) y cause irritation NTP? No xposure irritation ally Aggravated b edures nptomatically and ntacted area with o tions for Safe Material is Releas	No da data y Exposu supporti- copious a e Hand sed for S	Yes tta available for othe IARC Monogra No data re vely mounts of water. Iing and Use billed		Yes OSHA Regulation?	
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Address (Number, Street, City, State, 14676 Rothgeb Drive Rockville, MD 20850 Section II - Hazardous Ingred Hazardous Components [Specific Chemical Identity: Common Name(s)] This product contains no hazard Communication Standard. Section III - Physical/Chemic Boiling Point Vapor Pressure (mm Hg.) Vapor Density (AIR = 1) Solubility in Water Soluble Appearance and Odor Blue liquid, no o Section IV - Physical/Chemic Flash Point (Mthod Used) No data Extinguishing Media Dry chemical, ca	ients/Ider OSH/ Dus materia al Characc No data No data No data dor al Charac	Telephone Number for inform Date Prepared 12/17/9 Signature of Preparer (option ttify Information APEL ACGIH TLV Is as defined by the OSH teristics Melting Point Evaporation Rate (Butyl Acetate = 1) teristics	(301) 2 nation (301) 2 7 ial) Dther Limits ccommended A Hazard	51-5990 % (Optional) No data N/A No data	Route(s) of Entry: Health Hazards (Acute and Acute eye contact: May Carcinogenicity: None Signs and Symptoms of E: May cause skin or eye Medical Conditions Gener None reported Emergency First Aid Proc Treat syn Rinse con Steps to be Taken in case Rinse contacted area with Waste Disposal Method Observe all federal, state, Precautions to be Taken ir Avoid eye and skin contact Other Precautions None Section VIII - Control	Inhalatic Yes d Chronic) y cause irritation NTP? No xposure irritation ally Aggravated b edures nptomatically and ntacted area with o titions for Safe Material is Releas copious amounts and local regulation n Handling and Sto tt.	No da data y Exposu supporti a b Hand b Hand of water.	Yes tta available for othe IARC Monogra No data re vely mounts of water. ling and Use billed	phs?	Yes - OSHA Regulation? No data	
Address (Number, Street, City, State, 14676 Rothgeb Drive Rockville, MD 20850 Section II - Hazardous Ingred Hazardous Components [Specific Chemical Identity: Common Name(s)] This product contains no hazardo Communication Standard. Section III - Physical/Chemica Boiling Point Vapor Pressure (mm Hg.) Vapor Density (AIR = 1) Solubility in Water soluble Appearance and Odor Blue liquid, no o Section IV - Physical/Chemica Flash Point (Method Used) No data Extinguishing Media Dry chemical, cc Special Fire Fighting Procedures	ients/Ider OSH/ ous materia al Characci No data No data No data dor al Characci	Telephone Number for inform Date Prepared 12/17/9 Signature of Preparer (option titip Information APEL ACGIH TLV Read Read Is as defined by the OSH teristics Specific Gravity (H20 = 1) Melting Point Evaporation Rate (Butyl Acetate = 1) teristics Flammable Limits de, water spray or foam	(301) 2 hation (301) 2 7 hali) Dther Limits commended A Hazard) LEL No data	51-5990 % (Optional) No data N/A No data UEL No data	Route(s) of Entry: Health Hazards (Acute an Acute eye contact: May Carcinogenicity: None Signs and Symptoms of E: May cause skin or eye Medical Conditions Gener None reported Emergency First Aid Proc Treat syn Rinse con Section VII - Precau Steps to be Taken in case Rinse contacted area with Waste Disposal Method Observe all federal, state, Precautions to be Taken in Avoid eye and skin contact Other Precautions None Section VIII - Contro Respiratory Protection (Sg Ventilation Protective Cloves	Inhalatic Yes d Chronic) y cause irritation NTP? No xposure irritation ally Aggravated b edures nptomatically and ntacted area with o tions for Safe Material is Releas copious amounts and local regulated n Handling and Sto t.	No da data y Exposu supporti a b Hand b Hand of water.	Yes ta available for othe IARC Monogra No data re vely mounts of water. ling and Use iiiled rtridge respirator w Yes Yes	ith organic Special Other	Yes - OSHA Regulation? No data	
Address (Number, Street, City, State, 14676 Rothgeb Drive Rockville, MD 20850 Section II - Hazardous Ingred Hazardous Components [Specific Chemical Identity: Common Name(s)] This product contains no hazardo Communication Standard. Section III - Physical/Chemic Boiling Point Vapor Pressure (mm Hg.) Vapor Pressure (mm Hg.) Vapor Density (AIR = 1) Solubility in Water Solubile Appearance and Odor Blue liquid, no o Section IV - Physical/Chemic Flash Point (Method Used) No data Extinguishing Media Dry chemical, ca Special Fire Fighting Procedures Use agents suital breathing hazard	ients/Ider OSH/ ous materia al Characc No data No data No data dor al Characc dor al Characc	Telephone Number for inform Date Prepared 12/17/9 Signature of Preparer (option atify Information C PEL ACGIH TLV A PEL ACGIH TLV Resident as defined by the OSH teristics Specific Gravity (H ₂ 0 = 1) Melting Point Evaporation Rate (Butyl Acetate = 1) teristics Flammable Limits	(301) 2 hation (301) 2 7 hali) Pther Limits commended A Hazard A Hazard) LEL No data ep upwind, av	51-5990 % (Optional) No data N/A No data UEL No data	Route(s) of Entry: Health Hazards (Acute an Acute eye contact: May Carcinogenicity: None Signs and Symptoms of E: May cause skin or eye Medical Conditions Gener None reported Emergency First Aid Proc Treat syn Rinse con Section VII - Precau Steps to be Taken in case Rinse contacted area with Waste Disposal Method Observe all federal, state, Precautions to be Taken in Avoid eye and skin contact Other Precautions None Section VIII - Contro Respiratory Protection (Spiratory Protection (Spiratory Spiratory S	Inhalatic Yes d Chronic) y cause irritation NTP? No xposure irritation ally Aggravated b edures nptomatically and ntacted area with o titions for Safe Material is Releas copious amounts and local regulated h Handling and Sto ct.	No da data y Exposu supporti a b Hand b Hand of water.	Yes ta available for othe IARC Monogra No data re vely mounts of water. ling and Use iiiled rtridge respirator w Yes Yes	ith organic Special Other	Yes - OSHA Regulation? No data	
Address (Number, Street, City, State, 14676 Rothgeb Drive Rockville, MD 20850 Section II - Hazardous Ingred Hazardous Components [Specific Chemical Identity: Common Name(s)] This product contains no hazardo Communication Standard. Section III - Physical/Chemic Boiling Point Vapor Pressure (mm Hg.) Vapor Density (AIR = 1) Solubility in Water soluble Appearance and Odor Blue liquid, no o Section IV - Physical/Chemic Flash Point (Method Used) No data Extinguishing Media Dry chemical, ca Special Fire Fighting Procedures Use agents suital	ients/Ider OSH/ ous materia al Characc No data No data No data dor al Characc dor al Characc	Telephone Number for inform Date Prepared 12/17/9 Signature of Preparer (option htify Information ACGIH TLV Read as defined by the OSH teristics Specific Gravity (H20 = 1) Melting Point Evaporation Rate (Butyl Acetate = 1) teristics Flammable Limits le, water spray or foam of surrounding fire. Kee	(301) 2 hation (301) 2 7 hali) Pther Limits commended A Hazard A Hazard) LEL No data ep upwind, av	51-5990 % (Optional) No data N/A No data UEL No data	Route(s) of Entry: Health Hazards (Acute an Acute eye contact: May Carcinogenicity: None Signs and Symptoms of E: May cause skin or eye Medical Conditions Gener None reported Emergency First Aid Proc Treat syn Rinse con Section VII - Precau Steps to be Taken in case Rinse contacted area with Waste Disposal Method Observe all federal, state, Precautions to be Taken in Avoid eye and skin contact Other Precautions None Section VIII - Contro Respiratory Protection (Sg Ventilation Protective Cloves	Inhalatic Yes d Chronic) y cause irritation NTP? No xposure irritation ally Aggravated b edures potomatically and ntacted area with c tions for Safe Material is Releas copious amounts and local regulation h Handling and Sto ct.	No da data y Exposu supporti topious a B Hand seed for S of water. ons. oring emical ca eneral)	Yes Ita available for othe IARC Monogra No data Ire Vely mounts of water. Iing and Use Dilled Irtridge respirator with Yes Yes Yes Eye Protect uired	ith organic Special Other CtionSplast	Yes - OSHA Regulation? No data	

					Section V - Reactivit						
		laterial Safety Data S I to comply with OSHA's Haza		on	Stability	Unstable		Condit	tions to Avoi	id	
\ (edvotek "		CFR 1910.1200 Standard mu specific requirements.			Incompatibility	Stable	X		None		
					Incompatibility No						
IDENTITY (As Used on Label and List)		On Note: Blank spaces as applicable, or no inform be marked to indicate	re not permitted. If mation is available,	f any item is not , the space must	Hazardous Decomposition or Byproducts Sulfur oxides, and bromides						
Practice Gel Loa Section I	ung Soluti	be marked to indicate	that.		Hazardous	May Occur		Condi	itions to Avo	bid	
Manufacturer's Name		Emergency Telephone N	umber	054 5000	Polymerization	Will Not Occu			None		
EDVOTEK, Inc.		Tolophono Number for infor		251-5990	Section VI - Health H Route(s) of Entry:	lazard Data Inhala	tion?		Chin2		Ingestion? Ves
Address (Number, Street, City, State,	Zip Code)	Telephone Number for infor		251-5990	Yes Okin Yes ingotion Yes						
14676 Rothgeb Drive		Date Prepared 8/25/9	7		 Health Hazards (Acute and Chronic) Acute eye contact: May cause irritation. No data available for other routes. 						
Rockville, MD 20850		Signature of Preparer (optio	nal)		Carcinogenicity: NTP? IARC Monographs? OSHA Regulation? No data available						
					Signs and Symptoms of Ex		/ cause ski	n or eye	e irritation		
Section II - Hazardous Ingred	lients/Iden		Other Limits		Medical Conditions Genera						
Chemical Identity; Common Name(s)] OSHA PEL ACGIH TLV Recommended % (Optional)							5) Expoor	and N	None report	ted	
This product contains no hazardous r Standard.	materials as de	efined by the OSHA Hazard	Communicatio	on	Emergency First Aid Proce	110			lly and supp ts of water.		Rinse contacted area
Section III - Physical/Chemic	al Charact	teristics		_	Steps to be Taken in case				and Use		
Boiling Point	No data	Specific Gravity ($H_2^0 = T$	1)	No data	Wear eye and skin prot			-	with water		
Vapor Pressure (mm Hg.)	No data	Melting Point		No data	Waste Disposal Method						
		Evaporation Rate			Observe all federal, sta	te, and local reg	gulations.				
Vapor Density (AIR = 1)	No data	(Butyl Acetate = 1)		No data	Precautions to be Taken in	Handling and S	Storing				
Solubility in Water Soluble					Avoid eye and skin cor	-	-				
Appearance and Odor Blue liqu	uid, no odor				Other Precautions						
Section IV - Physical/Chemic	al Charac	teristics			None						
Flash Point (Method Used) No data		Flammable Limits	LEL	UEL	Section VIII - Contro						
Extinguishing Media			No data	No data	Respiratory Protection (Sp	ecify Type)					
Dry chemical,		de, water spray or foam			Ventilation	Local Exhaus			Yes	Special	None
Special Fire Fighting Procedures Us breathing hazardous sulfur oxides an			fire. Keep upw	ind, avoid	Protective Gloves	Mechanical (C	General)		Yes Eye Prot	Other	None
	la bronnaes.	wear SEBA.				Yes			Eyerio	ection	Splash proof goggles
Unusual Fire and Explosion Hazards	Unknown				Other Protective Clothing o	r Equipment	None re	equired			
					Work/Hygienic Practices		Avoid	eye and	skin conta	ct	
					Section V - Reactivity	/ Data					
	N May be use	Aterial Safety Data	Sheet	ion	Section V - Reactivity Stability	/ Data Unstable			ions to Avoid	ł	
	May be use	d to comply with OSHA's Haza OCFR 1910.1200 Standard m	ard Communicati	ion 1 for	Stability	Unstable Stable	X	Ν	None		
Carbtek®	May be use	d to comply with OSHA's Haza	ard Communicati	ion d for	Stability	Unstable Stable on, silver salt	s, hydros	N gen per	None roxide, ph	nenol, picr	ic acid gents.
IDENTITY (As Used on Label and List)	May be user Standard. 29	d to comply with OSHA's Haza 9 CFR 1910.1200 Standard m specific requirements. Note: Blank spaces a applicable, or no infor	ard Communicati nust be consulted are not permitted. rmation is available	d for	Stability Incompatibility Copper, ir Formaldehyde, ether, al Hazardous Decomposition or E	Unstable Stable on, silver salt cohol, nitrog syproducts	s, hydrog en oxide	N gen per , strong	None roxide, ph g bases, o	nenol, picr xidizing a	gents.
Enzyme Reaction	May be user Standard. 29	d to comply with OSHA's Haza CFR 1910.1200 Standard m specific requirements. Note: Blank spaces a	ard Communicati nust be consulted are not permitted. rmation is available	d for	Stability Incompatibility Copper, ir Formaldehyde, ether, al Hazardous Decomposition or E Toxic gases: Carbon m Hazardous	Unstable Stable on, silver salt cohol, nitrog syproducts	s, hydrog en oxide	N gen per , strong ide, nit	None roxide, ph g bases, o rogen oxi tions to Avoi	ienol, picr xidizing a ides, chlor	gents.
	May be user Standard. 29	d to comply with OSHA's Haza 9 CFR 1910.1200 Standard m specific requirements. Note: Blank spaces a applicable, or no infor	ard Communicati nust be consulted are not permitted. mation is available that.	d for If any item is not e, the space must	Stability Incompatibility Copper, ir Formaldehyde, ether, al Hazardous Decomposition or E Toxic gases: Carbon m Hazardous Polymerization	Unstable Stable on, silver salt cohol, nitrog syproducts onoxide, cart May Occur Will Not Occur	s, hydrog en oxide,	N gen per , strong ide, nit	None roxide, ph g bases, o rogen oxi	ienol, picr xidizing a ides, chlor	gents.
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				Section V - Reactivity Data							
Material Safety Data Sheet May be used to comply with OSHA's Hazard Communication				Stability	Unstable Conditions to Avoid						
Standard. 29 CFR 1910.1200 Standard must be consulted for						Stable	Х	Ne	one		
specific requirements.					Incompatibility Strong oxidizing agents						
IDENTITY (As Used on Label and List) Note: Blank spaces are not permitted. If any item is not				Hazardous Decomposition or B							
DNA Blue InstaStain	applicable, or no information is available, the space must be marked to indicate that.			Toxic fumes of Carbon monoxide, Carbon dioxide, nitrogen oxides, sulfur oxides, hydrogen, chloride gas							
Section I					Hazardous Polymerization	May Occur		Conditions to Avo	d		
Manufacturer's Name	E	Emergency Telephone Number			Will Not Occur A None						
EDVOTEK, Inc.		(301) 251-5990			Section VI - Health H						
Address (Number, Street, City, State, Zip C	Code) T	Telephone Number for information (301) 251-5990			Route(s) of Entry:	Inhalatio	n? Yes	Skin?	Yes	Ingestion? Yes	
14676 Rothgeb Drive	7	Date Prepared 03-01-2000			Health Hazards (Acute and Skin: May cause skin	n irritation I	Eyes: Maj	y cause eye irritat		Inhalation: Cyanosis	
Rockville, MD 20850		ignature of Preparer (optiona	al)		Carcinogenicity: Meets criteria for pro	NTP?	4:1	IARC Monogr		OSHA Regulation?	
		ignatare er rieparer (optione	,		Signs and Symptoms of Exp	ocuro			47.30420).82	
Section II - Hazardous Ingredients	ts/Identif	v Information			oigna and oymptoma of Exp	No da	ıta availab	ole			
Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA P	Ot	her Limits	% (Optional)	Medical Conditions General	lly Aggravated by	y Exposur	e No data avai	lable		
Methylene Blue	USHA F	EL ACGIH TLV Rec	commended	% (Optional)	Emergency First Aid Procee	dures Treat sv	mptomatio	cally			
3.7 Bis (Dimethylamino) Phenothiazin 5 IUN	M Chlorid	e No data available				ficat sy	inpromativ	carry			
CAS # 61-73-4					Section VII - Precauti	ions for Safe	Handl	ing and Lloa			
Section III - Physical/Chemical Ch	haracter	istics									
Boiling Point No	o data	Specific Gravity (H ₂ 0 = 1)		No data	Steps to be Taken in case Material is Released for Spilled Ventilate area and wash spill site						
Vapor Pressure (mm Hg.) No	o data	Melting Point		No data	Waste Disposal Method Mix material with a combustible solvent and burn in chemical						
Vapor Density (AIR = 1) No	o data	Evaporation Rate (Butyl Acetate = 1)		No data	incinerator equipped with			. Check local and	state regu	ilations.	
Solubility in Water Soluble - cold		(Duty) / toetate = 1)			Precautions to be Taken in H	0	•				
Solubility in Water Soluble - cold					Keep tightly closed. Stor	re in cool, dry pl	ace				
Appearance and Odor Chemical bound to	paper				Other Precautions						
					None						
Section IV - Physical/Chemical C			LEL	UEL	Section VIII - Control Measures						
Flash Point (Method Used) No data available		Flammable Limits	LEL No data	UEL No data	Respiratory Protection (Specify Type) MIOSH/OSHA approved, SCBA						
Extinguishing Media Water spray, carb	bon dioxide	, dry chemical powder, alc	ohol or polvm	er foam	Ventilation	Local Exhaust			Special		
Special Fire Fighting Procedures			1.7			Mechanical (Ge	neral)	Required	Other		
Self contained breathing apparatus	is and prote	ctive clothing to prevent co	ontact with skin	n and eyes	Protective Gloves Rubbe	er -		Eye Prote	ection (Chem. safety goggles	
Unusual Fire and Explosion Hazards					Other Protective Clothing or	Equipment	Rubber b	poots			
Emits toxid fumes under fire conditions					Work/Hygienic Practices						