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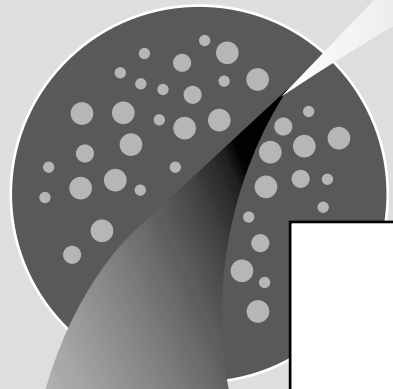

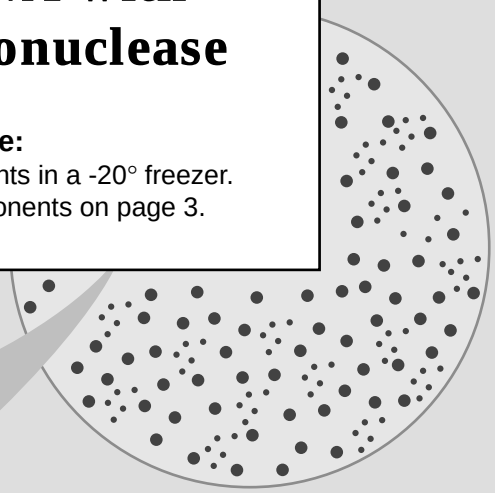

212

EDVO-Kit #

Cleavage of Lambda DNA with *Eco* RI Endonuclease

Storage:
Store biological components in a -20° freezer.
See Experiment Components on page 3.

Experiment Objective:

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

Major Section Headings

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Experiment Components

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA.

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
A	Lambda phage DNA	Freezer
B	Concentrated Restriction Enzyme Reaction Buffer	Freezer
C	EDVOTEK enzyme grade water	Freezer
D	*Eco* RI Dryzymes™ endonuclease	Room temp with desiccant
E	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

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.

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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

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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.

Restriction Enzyme	Organism
Bgl I	*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

Figure 1

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.

```
      ↓                    
5'-GAATTC-3'        5'-G    AATTC-3'
3'-CTTAAG-5'        3-CTTAA     G-5'
        ↑
```

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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.

```
      ↓
5'-GGCC-3'          5'-GG    CC-3'
3'-CCGG-5'          3'-CC    GG-5'
      ↑
```

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

```
    ↓
5'-CPyCGPuG-3'      (Py = pyrimidine = C or T and
3'-GPuGCPyC-5'       Pu = purine = G or A)
          ↑
```

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:

```
          ↓
5'-GCCNNNNNGGC-3'     (N = A, G, C or T)
3'-CGGNNNNNCCG-5'
          ↑
```

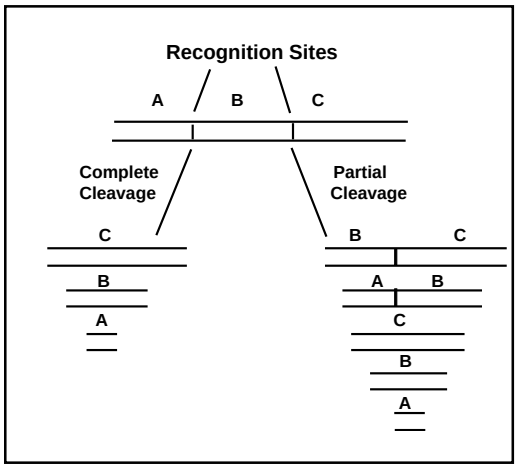

Figure 2

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.

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BACKGROUND INFORMATION

Background Information, continued

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.

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

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 **cos** 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.

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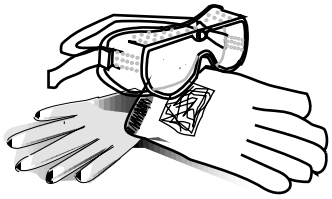
EXPERIMENTAL PROCEDURES

EXPERIMENT OBJECTIVE

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

LABORATORY SAFETY



WEAR SAFETY GOGGLES AND GLOVES

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.

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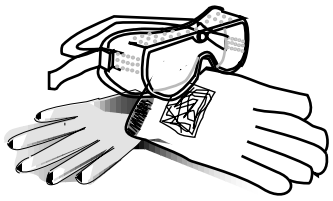
EXPERIMENTAL PROCEDURES

Restriction Enzyme Digestion

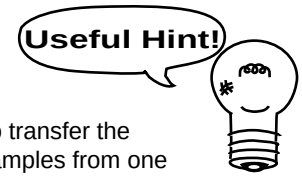


WEAR SAFETY GOGGLES AND GLOVES

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 FRESH 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.



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.

SETTING UP THE DIGESTION REACTION

4. With a FRESH 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 µl 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 →	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

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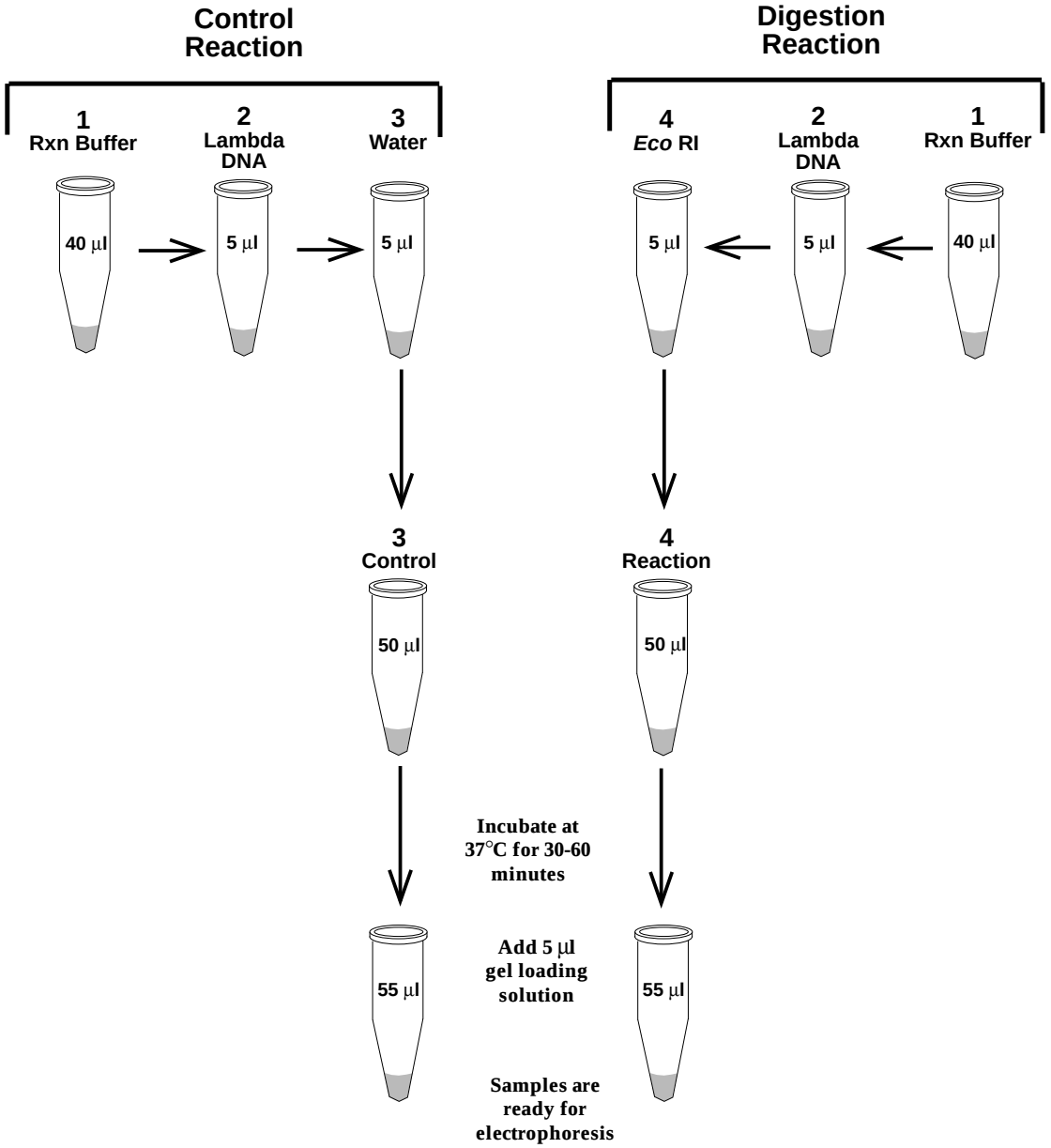
EXPERIMENTAL PROCEDURES

Restriction Enzyme Digestion, continued

SCHEMATIC FLOW DIAGRAM OF RESTRICTION ENZYME DIGEST



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

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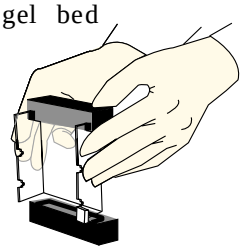
EXPERIMENTAL PROCEDURES

Agarose Gel Preparation

PREPARING THE GEL BED

Using 7 x 7 cm Gel Beds

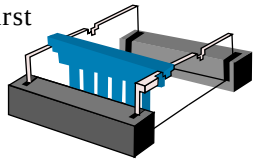
1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.



   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.



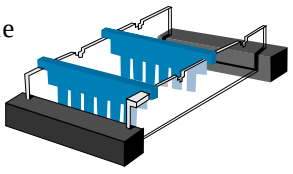
Using the 7 x 15 cm Gel Bed for Two Gels

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.



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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.

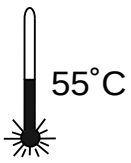
5. With a marking pen, indicate the level of the solution volume on the outside of the flask.

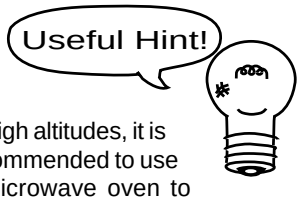
6. Heat the mixture to dissolve the agarose powder. The final solution should be clear (like water) without any undissolved particles.

   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.

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





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

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EXPERIMENTAL PROCEDURES

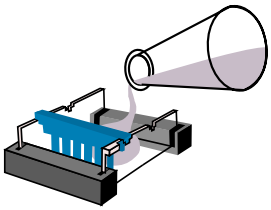
Agarose Gel Preparation, continued

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 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.



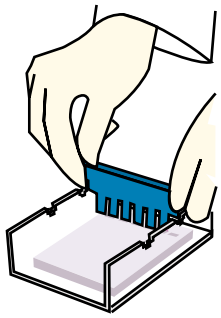
9. Pour the cooled agarose solution into the bed. Make sure the bed is on a level surface.

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

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.

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: Electrophoresis (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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EXPERIMENTAL PROCEDURES

Agarose Gel Preparation, continued

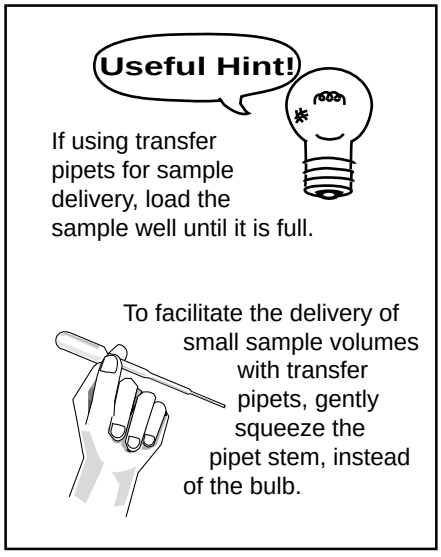
15. Make sure the gel is completely covered with buffer. The agarose gel is sometimes called a "submarine gel" because it is submerged under buffer for sample loading and electrophoretic separation.

16. Load samples in wells and conduct electrophoresis according to experiment instructions starting on page 15.

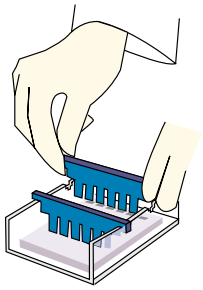
Sample Delivery and Practice Gel Loading

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.

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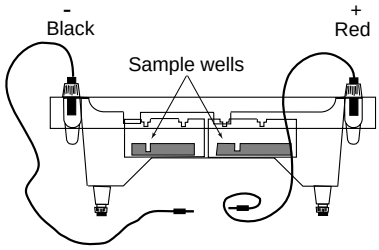
EXPERIMENTAL PROCEDURES

Conducting Agarose Gel Electrophoresis

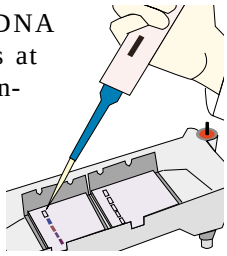
Reminder:

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



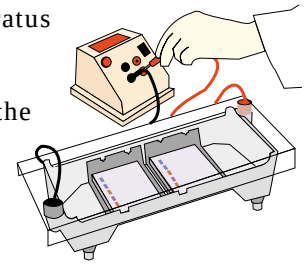
1. Heat the samples, including the Standard DNA fragments (Component E) for two minutes at 65°C. Allow the samples to cool for a few minutes.
2. Load the first well of each row of samples with 40 µl of the Standard DNA fragments (E).
3. Load 40 µl of the reaction and control samples into separate wells.

   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*	Recommended Time	
	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.

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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™.

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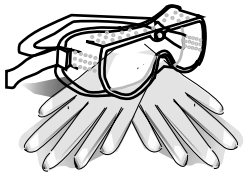
EXPERIMENTAL PROCEDURES

Staining & Visualization of DNA

NEW
Stain DNA with DNA Blue InstaStain™
Patents Pending



WEAR SAFETY GOGGLES AND GLOVES

DNA BLUE INSTASTAIN™

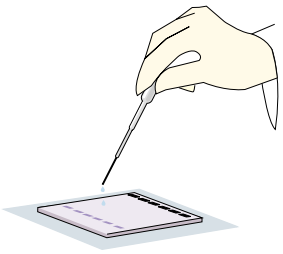
Many EDVOTEK electrophoresis experiments now feature **InstaStain™**, a new proprietary staining method for staining DNA separated on agarose gels. Based on state-of-the-art technology, this experiment includes DNA Blue InstaStain™, which is a safe, quick, non-liquid method for staining gels. It minimizes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

Advantages of DNA Blue InstaStain™ vs. Liquid Staining

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

Staining with DNA Blue InstaStain™

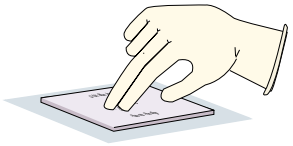
1. After electrophoresis is completed, place the gel on a flat surface. Moisten the gel with several drops of electrophoresis buffer.



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.

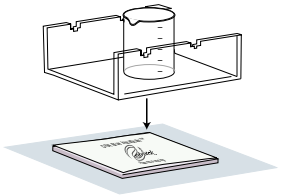
   Do this several times.



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 InstaStain™ to sit for 15 minutes.



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

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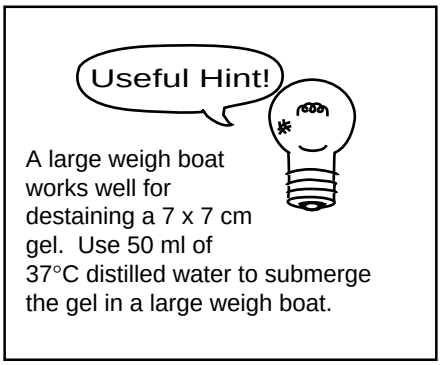
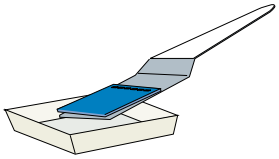
EXPERIMENTAL PROCEDURES

Staining & Visualization of DNA, cont.

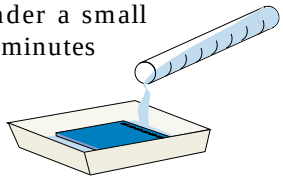


Destaining and Visualization of DNA



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

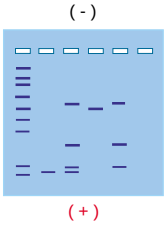
6. Conduct destaining with distilled water that has been warmed to 37°C.



   - 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.

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

7. After the first destain, the larger DNA bands will be visible as dark blue bands against a lighter blue background. When completely 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.

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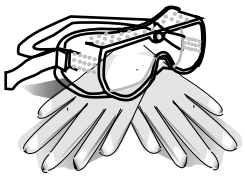
EXPERIMENTAL PROCEDURES

Staining & Visualization of DNA, cont.



WEAR SAFETY GOGGLES AND GLOVES

After electrophoresis, each group should remove a small slice, or make a small hole in a designated corner of their gel, to facilitate identification after staining and destaining.

Dilution of Methylene Blue Plus™ stain:

Dilute the 10x stain by mixing 1 part stain with 9 parts distilled or deionized water.

TRADITIONAL LIQUID STAINING WITH METHYLENE BLUE PLUS™

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.

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

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

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.

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EXPERIMENTAL PROCEDURES

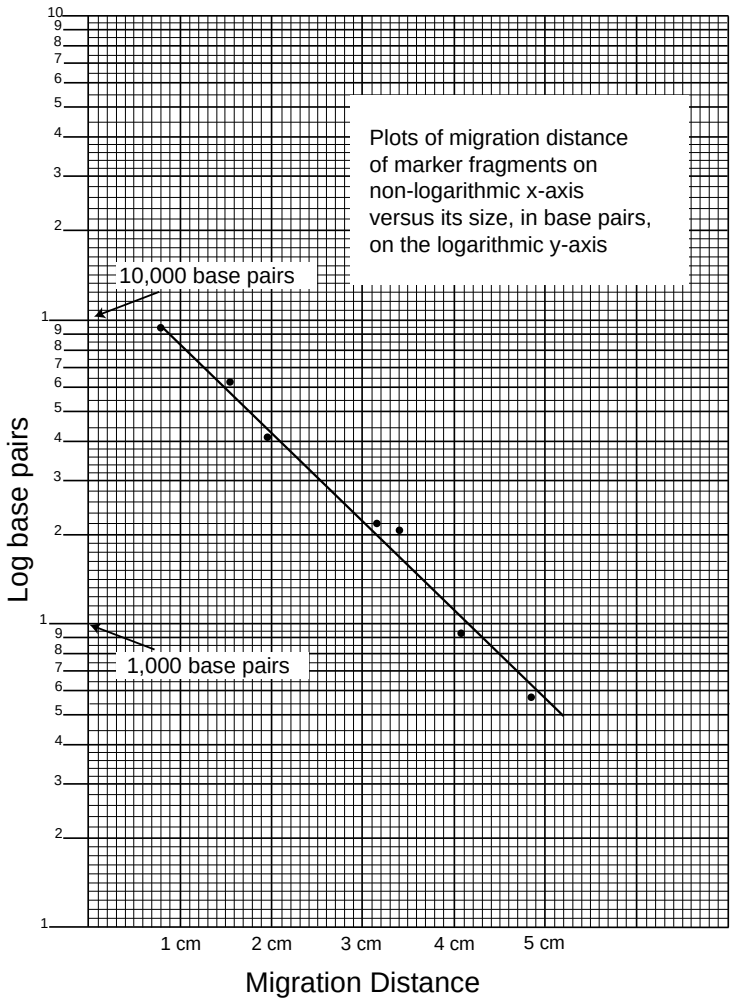
Size Determination of DNA Fragments

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 ± 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).

Figure 3



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 y-axis 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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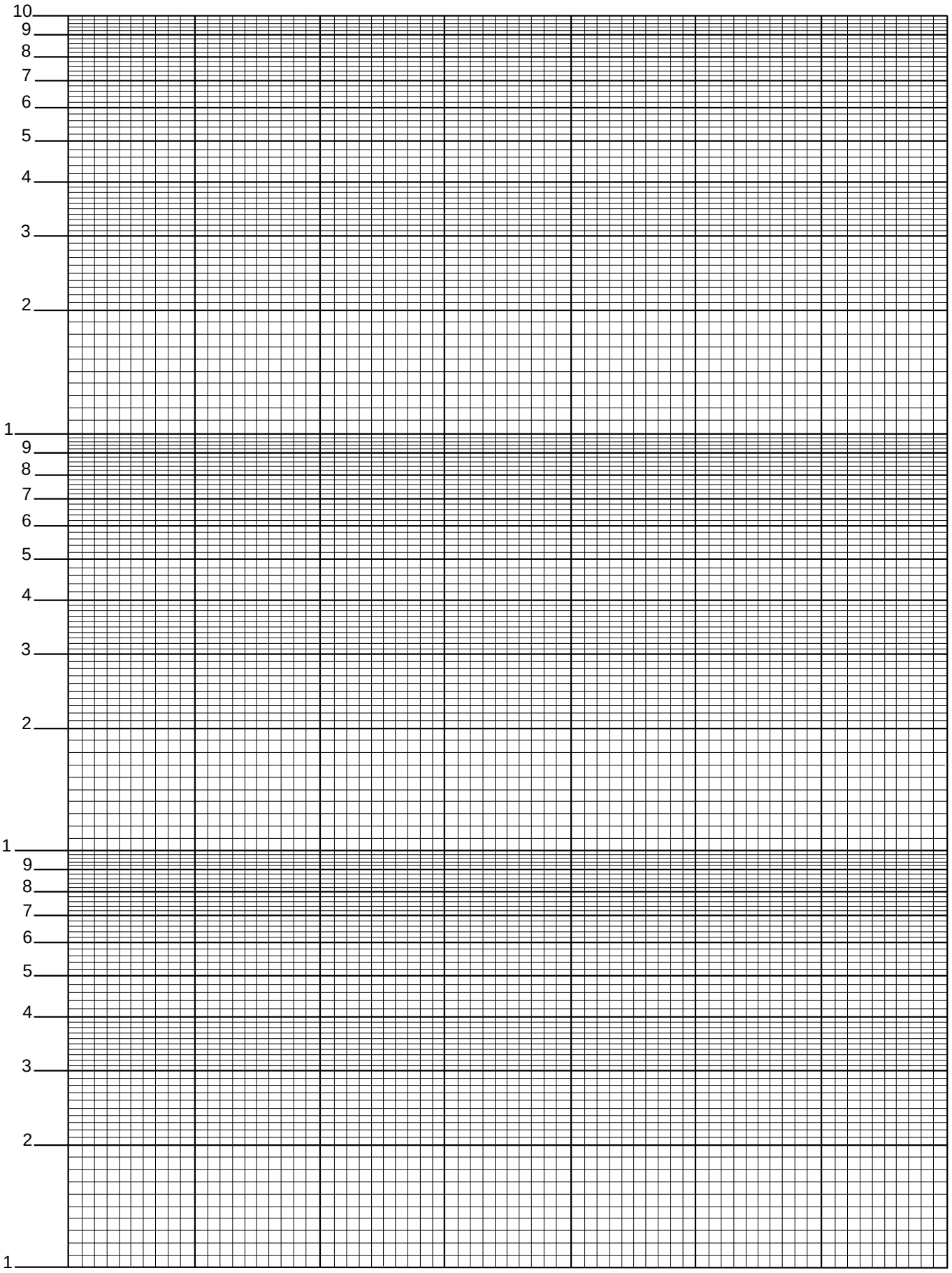
10
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Material Safety Data Sheet

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: 8/25/97

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_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Insoluble - cold

Appearance and Odor: White powder, no odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

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

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

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

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		None
	Stable	X	

Incompatibility: No data available

Hazardous Decomposition or Byproducts:

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): 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: 09/15/97

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

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

Section III - Physical/Chemical Characteristics

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

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

Appearance and Odor: Clear, liquid, slight vinegar odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

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

Extinguishing Media: Use extinguishing media appropriate for surrounding fire.

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

Unusual Fire and Explosion Hazards: None identified

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		None
	Stable	X	

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): None

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

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

Medical Conditions Generally Aggravated by Exposure: None

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

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

Section VII - Precautions for Safe Handling and Use

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

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

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

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

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

Protective Gloves: Yes Eye Protection: Safety goggles

Other Protective Clothing or Equipment: None

Work/Hygienic Practices: None

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): **Methylene Blue Plus ™**

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: 8/25/97

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)
Methylene Blue				
3.7 Bis (Dimethylamino) Phenothiazin 5 IUM Chloride	No data available			
CAS # 61-73-4				

Section III - Physical/Chemical Characteristics

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

Solubility in Water: Soluble - cold

Appearance and Odor: Blue solution, no odor

Section IV - Physical/Chemical Characteristics

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

Extinguishing Media: Water spray, carbon dioxide, dry chemical powder, alcohol or polymer foam

Special Fire Fighting Procedures: Self contained breathing apparatus and protective clothing to prevent contact with skin and eyes

Unusual Fire and Explosion Hazards: Emits toxid fumes under fire conditions

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	None

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Toxic fumes of Carbon monoxide, Carbon dioxide, nitrogen oxides, sulfur oxides, hydrogen, chloride gas

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	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): Skin: May cause skin irritation Eyes: May cause eye irritation Inhalation: Cyanosis

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?
Meets criteria for proposed OSHA medical records rule PEREAC 47.30420.82

Signs and Symptoms of Exposure: No data available

Medical Conditions Generally Aggravated by Exposure: No data available

Emergency First Aid Procedures: Treat symptomatically

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Ventilate area and wash spill site

Waste Disposal Method: Mix material with a combustible solvent and burn in chemical incinerator equipped with afterburner and scrubber. Check local and state regulations.

Precautions to be Taken in Handling and Storing: Keep tightly closed. Store in cool, dry place

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): MIOSH/OSHA approved, SCBA

Ventilation			
	Local Exhaust		Special
	Mechanical (General)	Required	Other

Protective Gloves: Rubber Eye Protection: Chem. safety goggles

Other Protective Clothing or Equipment: Rubber boots

Work/Hygienic Practices:

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): Gel loading solution concentrate, 10x

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

Section I

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

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

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

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

Date Prepared: 12/17/97

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

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

Section III - Physical/Chemical Characteristics

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

Solubility in Water: soluble

Appearance and Odor: Blue liquid, no odor

Section IV - Physical/Chemical Characteristics

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

Extinguishing Media: Dry chemical, carbon dioxide, water spray or foam

Special Fire Fighting Procedures: Use agents suitable for type of surrounding fire. Keep upwind, avoid breathing hazardous sulfur oxides and bromides. Wear SCBA.

Unusual Fire and Explosion Hazards: Unknown

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	None

Incompatibility: None known

Hazardous Decomposition or Byproducts: Sulfur oxides and bromides

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

Section VI - Health Hazard Data

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

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

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

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: Rinse contacted area with copious amounts of 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): Chemical cartridge respirator with organic vapor cartridge.

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

Protective Gloves: yes Eye Protection: Splash proof goggles

Other Protective Clothing or Equipment: None required

Work/Hygienic Practices: Do not ingest. Avoid contact with skin, eyes and clothing. Wash thoroughly after handling.

Material Safety Data Sheet

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

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

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

Section I

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

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

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

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

Date Prepared: 8/25/97

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

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

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

Section III - Physical/Chemical Characteristics

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

Solubility in Water: Soluble

Appearance and Odor: Blue liquid, no odor

Section IV - Physical/Chemical Characteristics

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

Extinguishing Media: Dry chemical, carbon dioxide, water spray or foam

Special Fire Fighting Procedures: Use agents suitable for type of surrounding fire. Keep upwind, avoid breathing hazardous sulfur oxides and bromides. Wear SCBA.

Unusual Fire and Explosion Hazards: Unknown

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		None
	Stable	X	

Incompatibility: None

Hazardous Decomposition or Byproducts: Sulfur oxides, and bromides

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

Section VI - Health Hazard Data

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

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

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

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

Medical Conditions Generally Aggravated by Exposure: None reported

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

Section VII - Precautions for Safe Handling and Use

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

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

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

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

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

Protective Gloves: Yes Eye Protection: Splash proof goggles

Other Protective Clothing or Equipment: None required

Work/Hygienic Practices: Avoid eye and skin contact

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): Enzyme Reaction 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: 12/17/97

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

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

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

Section III - Physical/Chemical Characteristics

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

Solubility in Water: soluble

Appearance and Odor: Clear liquid, no odor, dry chemical, carbon dioxide, water spray or foam.

Section IV - Physical/Chemical Characteristics

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

Extinguishing Media: Use extinguishing media appropriate to surrounding fire

Special Fire Fighting Procedures: Remove container from fire if possible.

Unusual Fire and Explosion Hazards: May produce toxic gases

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		None
	Stable	X	

Incompatibility: Copper, iron, silver salts, hydrogen peroxide, phenol, picric acid Formaldehyde, ether, alcohol, nitrogen oxide, strong bases, oxidizing agents.

Hazardous Decomposition or Byproducts: Toxic gases: Carbon monoxide, carbon dioxide, nitrogen oxides, chlorine, hydrogen chloride

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): Toxicity has not been quantified. Sensitivity reactions (allergic) may occur by skin penetration including anaphylactic shock.

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

Signs and Symptoms of Exposure: May cause irritation to skin/eye, mucous membranes and upper respiratory tract.

Medical Conditions Generally Aggravated by Exposure: Respiratory conditions

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: Mop up with absorbant material. Dispose of properly.

Waste Disposal Method: Mix with vermiculite and dry caustic, wrap in paper and burn in a chemical incinerator equipped with afterburner and scrubber. Ignite in presence of sodium carbonate and slaked lime (CaOH)

Precautions to be Taken in Handling and Storing: Wear protective gear to avoid skin/eye contact

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator with organic vapor cartridge.

Ventilation				
	Local Exhaust	Yes	Special	None
	Mechanical (General)	No	Other	Process Encl. Vent. Sys.

Protective Gloves: yes Eye Protection: Splash proof goggles

Other Protective Clothing or Equipment: Protection to avoid skin contact

Work/Hygienic Practices: Do not ingest. Avoid contact with skin, eyes and clothing. Wash thoroughly after handling.

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)
DNA Blue InstaStain ™

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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
03-01-2000

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)
Methylene Blue				
3.7 Bis (Dimethylamino) Phenothiazin 5 IUM Chloride	No data available			
CAS # 61-73-4				

Section III - Physical/Chemical Characteristics

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

Solubility in Water: Soluble - cold

Appearance and Odor: Chemical bound to paper

Section IV - Physical/Chemical Characteristics

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

Extinguishing Media: Water spray, carbon dioxide, dry chemical powder, alcohol or polymer foam

Special Fire Fighting Procedures: Self contained breathing apparatus and protective clothing to prevent contact with skin and eyes

Unusual Fire and Explosion Hazards: Emits toxid fumes under fire conditions

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	None

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Toxic fumes of Carbon monoxide, Carbon dioxide, nitrogen oxides, sulfur oxides, hydrogen, chloride gas

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	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)
Skin: May cause skin irritation Eyes: May cause eye irritation Inhalation: Cyanosis

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?
Meets criteria for proposed OSHA medical records rule PEREAC 47.30420.82

Signs and Symptoms of Exposure: No data available

Medical Conditions Generally Aggravated by Exposure: No data available

Emergency First Aid Procedures: Treat symptomatically

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled
Ventilate area and wash spill site

Waste Disposal Method: Mix material with a combustible solvent and burn in chemical incinerator equipped with afterburner and scrubber. Check local and state regulations.

Precautions to be Taken in Handling and Storing
Keep tightly closed. Store in cool, dry place

Other Precautions
None

Section VIII - Control Measures

Respiratory Protection (Specify Type): MIOSH/OSHA approved, SCBA

Ventilation		
	Local Exhaust	Special
	Mechanical (General) Required	Other

Protective Gloves: Rubber Eye Protection: Chem. safety goggles

Other Protective Clothing or Equipment: Rubber boots

Work/Hygienic Practices