EDVOTEK®

The Biotechnology Education Company ®

Revised and Updated

EDVO-Kit

104

Size Determination of DNA Restriction Fragments

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

The objective of this experiment module is to develop an understanding of principles involved in estimating the size of unknown DNA fragments by agarose gel electrophoresis.

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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.

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA. None of the experiment components are derived from human sources.

Experiment Components

DNA samples are stable at room temperature. However, if the experiment will not be conducted within one month of receipt, it is recommended that the DNA samples be stored in the refrigerator.

DNA samples do not require heating prior to gel loading.

Note: If you ordered Experiment #104-Q, the experiment components include InstaStain® Ethidium bromide instead of FlashBlue™ and InstaStain® Blue DNA stains.

DNA SAMPLES FOR ELECTROPHORESIS

The DNA samples for electrophoresis in experiment 104 are packaged in one of the following ways:

- Pre-aliquoted Quickstrip™ connected tubes, which contain:
  A and D Standard DNA Fragments
  B and E Unknown DNA 1
  C and F Unknown DNA 2
  G and H blank

OR

- Individual 1.5 ml (or 0.5 ml) microcentrifuge tubes, which contain:
  A Standard DNA Fragments
  B Unknown DNA 1
  C Unknown DNA 2

REAGENTS & SUPPLIES

- UltraSpec-Agarose™ powder
- Concentrated electrophoresis buffer
- FlashBlue™ DNA Stain
- InstaStain® Blue cards
- Practice Gel Loading Solution
- 1 ml pipet
- Microtipped Transfer Pipets

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips
- Balance
- Microwave, hot plate or burner
- Pipet pump
- 250 ml flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Small plastic trays or large weigh boats (for gel destaining)
- DNA visualization system (white light)
- Distilled or deionized water

Background Information

Size determination of DNA fragments is essential to DNA mapping and analyzing restriction enzyme cleavage patterns. Restriction enzymes are endonucleases that cleave both strands of DNA at very specific sequences within DNA. Locations of their cleavage sites are important for DNA fingerprinting, determination of genetic diseases and for DNA analysis.

Agarose gel electrophoresis is a convenient analytical method for determining the size of DNA molecules in the range of 500 to 30,000 base pairs. Samples of DNA are delivered in wells made in an agarose gel, which is placed in an electrophoresis chamber containing a buffer solution and electrodes. Direct current (D.C.) is applied from a power source. Since DNA is negatively charged at neutral pH, it will migrate through the gel towards the positive electrode. The agarose gel consists of microscopic pores that act as a molecular sieve that separates DNA molecules according to their size and shape. The migration rate of DNA molecules of the same shape is inversely proportional to their size. This results in smaller DNA molecules to migrate faster through the gel. The charge to mass ratio is the same for different sized DNA molecules.

Nucleotides in DNA are linked together by negatively charged phosphodiester bonds. For every base pair (average molecular weight of approximately 660) there are two charged phosphodiester linkages. Therefore, negative charges in DNA is accompanied by approximately the same mass. The absolute amount of charge in DNA is not a critical factor in the separation process. Separation occurs because smaller molecules pass through the gel pores more easily than larger ones (i.e., the gel is sensitive to the physical size of the molecule). DNA fragment migration rate is inversely proportional to the log10 of its size in base pairs.

Quick Reference:

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

23130	9416	6557
4361	3000	2322
2027	725	570

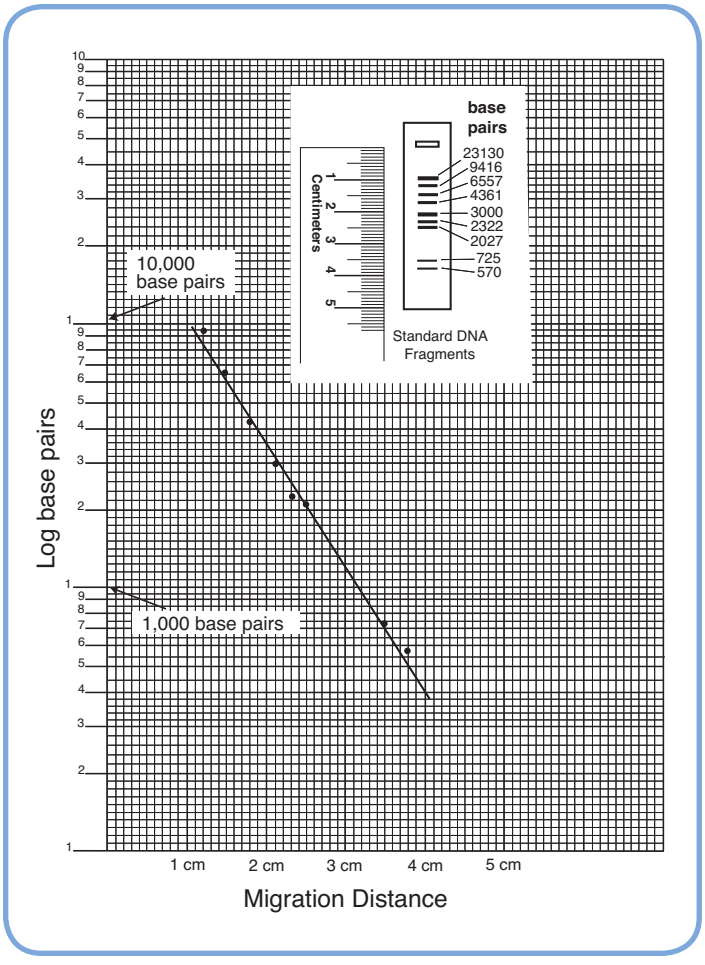
In this experiment, DNA fragments of unknown size and Standard DNA fragments are submitted to electrophoretic separation. The unknown DNA fragments will migrate through the gel according to their respective sizes and relative to the Standard DNA fragments. After electrophoresis, the gel is stained and the DNA bands are visualized. The migration distances of the known and unknown fragments are measured and plotted on semi-log graph paper according to their size on the y-axis versus the migration distance on the x-axis. The size of the fragments on the y-axis are expressed as the log of the number of base pairs. This allows the data to be plotted as a straight line. The DNA fragments of known size (Standard DNA fragments) are used to plot a standard curve. The migration distance of the unknown DNA fragments are estimated by extrapolation from the standard curve.

Background Information

The standard fragments are used to make a standard curve by plotting their size on the y-axis versus the migration distance on the x-axis. The size of the fragments on the y-axis are expressed as the log of the number of base pairs they contain or the log of their molecular weight. Most of the plotted data obtained from the markers will yield a straight line. The migration distance of the unknown DNA fragment(s) are found on the X-axis and their size is estimated from the standard curve.

After determining the size of the DNA fragments generated by single and combinations of restriction enzymes, a DNA map is constructed as previously described.

In this experiment, you will determine the relative locations of three restriction enzyme cleavage sites on a circular plasmid DNA. The plasmid has been cleaved with two restriction enzymes. Enzyme 1 cleaves the plasmid once. Assume that the Enzyme 1 site is at position 0. Enzyme 2 cuts the plasmid twice. The objective is to calculate the distances in base pairs between the points of cleavage and to determine whether the Enzyme 1 site is in between the Enzyme 2 sites.



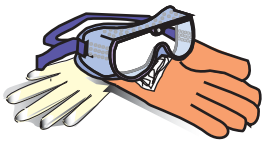
Experiment Overview and General Instructions

Experiment Procedure

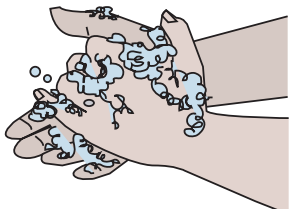
EXPERIMENT OBJECTIVE:

The objective of this experiment module is to develop an understanding of principles involved in estimating the size of unknown DNA fragments by agarose gel electrophoresis.

LABORATORY SAFETY



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



LABORATORY NOTEBOOK RECORDINGS:

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

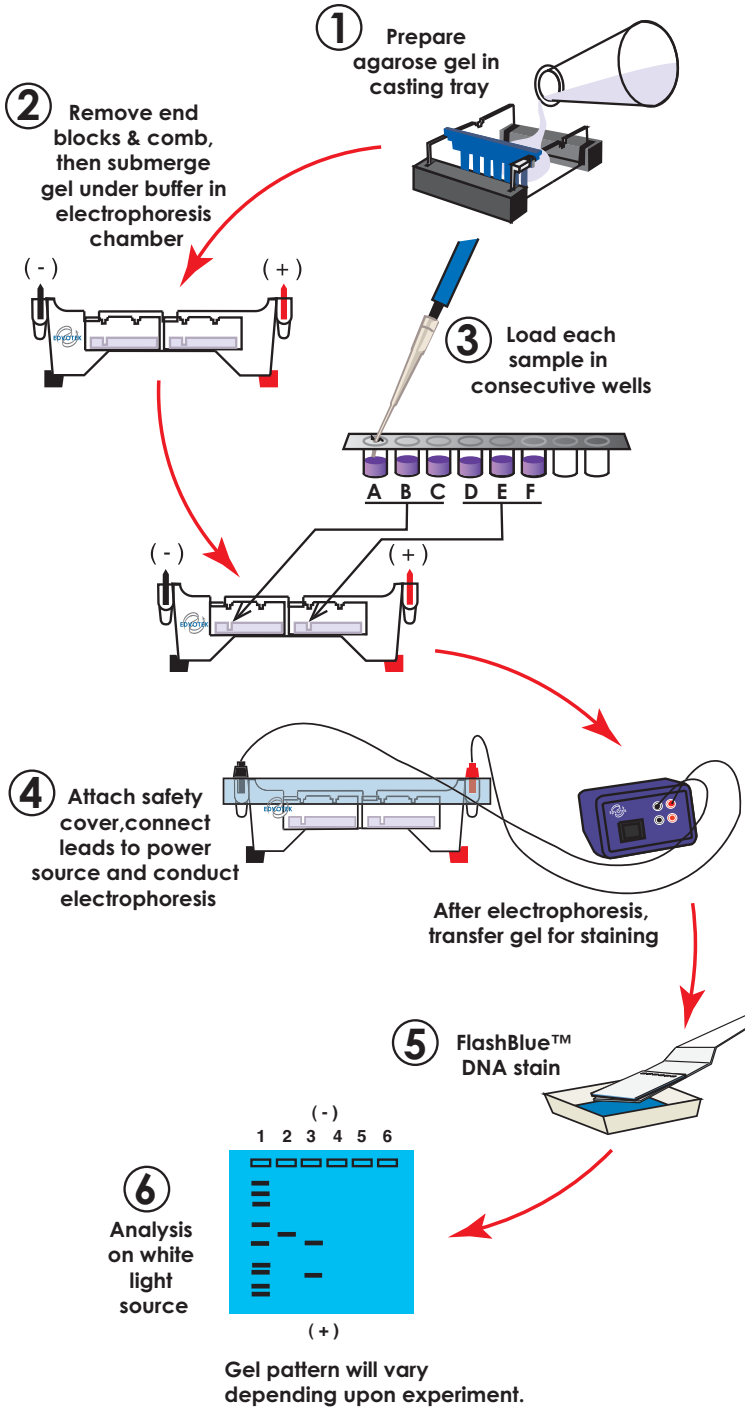
During the Experiment:

- Record (draw) your observations, or photograph the results.

Following the Experiment:

- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

Experiment Overview: Flow Chart



Agarose Gel Electrophoresis

Wear Gloves & goggles

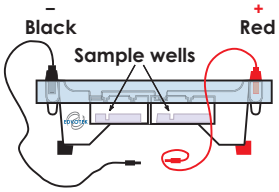
For gels to be stained with FlashBlue™ or InstaStain® Blue, prepare gels according to Appendix A.

For gels to be stained with InstaStain® Ethidium bromide, prepare gels according to Appendix B.

Step-by-step guidelines for agarose gel preparation are summarized in Appendix D.

Reminders:

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.



Prepare the Gel

1. Prepare an agarose gel with specifications summarized below. Your instructor will specify which DNA stain you will be using.

- Agarose gel concentration required: 0.8%
- Recommended gel size: 7 x 7 cm or 7 x 14 cm (two gels)
- Number of sample wells required: 3
- Placement of well-former template: first set of notches (7 x 7 cm) first & third set of notches (7 x 14 cm)

Load the Samples

2. Load the DNA samples in tubes A - C (or D - F) into the wells in consecutive order.

- For gels to be stained with FlashBlue™ or InstaStain® Blue, load wells with 35 - 38 µl.
- For gels to be stained with InstaStain® Ethidium Bromide, load wells with 18 - 20 µl.

Lane	Tube	
1	A or D	Standard DNA Fragments
2	B or E	Unknown 1
3	C or F	Unknown 2

Run the Gel

3. After DNA samples are loaded, connect the apparatus to the D.C. power source and set the power source at the required voltage.

4. Check that current is flowing properly - you should see bubbles forming on the two platinum electrodes. Conduct electrophoresis for the length of time specified by your instructor.

5. After electrophoresis is completed, proceed to DNA staining and visualization. Refer to Appendix E, F, G, or H for the appropriate staining instructions.

6. Document the results of the gel by photodocumentation.

   Alternatively, place transparency film on the gel and trace it with a permanent marking pen. Remember to include the outline of the gel and the sample wells in addition to the migration pattern of the DNA bands.

Experiment Procedure

Size Determination of DNA Restriction Fragments

This exercise focuses on the first step for mapping DNA restriction sites, which is to determine the size of "unknown" DNA fragments generated after electrophoresis. The assignment of sizes for DNA fragments separated by agarose gel electrophoresis can have ± 10% margin of error. The sizes of the "unknowns" will be extrapolated by their migration distances relative to the Standard DNA Fragments (Sample A or D), for which the fragment sizes are known.

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 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.

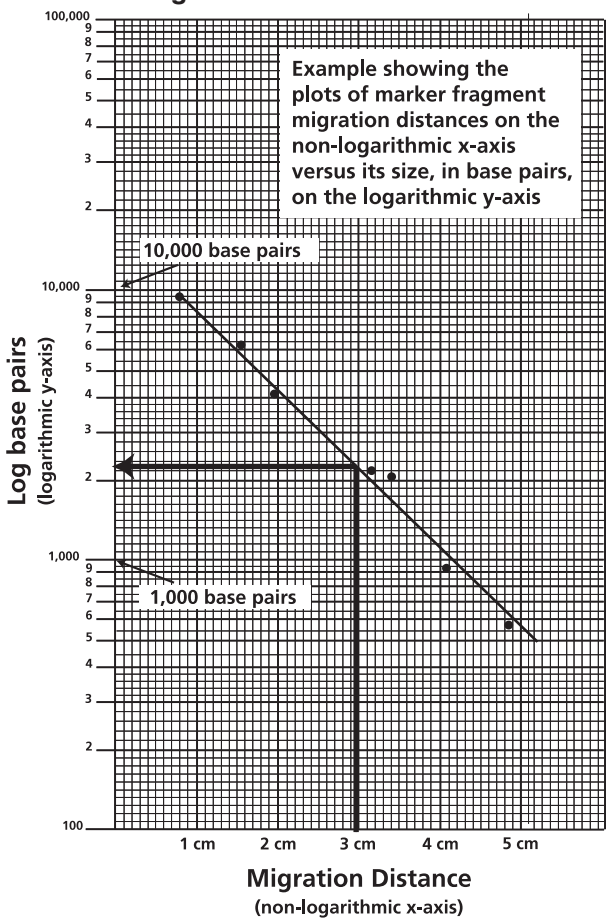
4. Draw the best average straight line through all the points. The line should have approximately equal numbers of points scattered on each side of the line. Some points may be right on the line (see Figure 1 for an example).

5. Measure the migration distance of each of the "unknown" fragments from samples B and C (or E and F).

6. Using the graph of the Standard DNA fragments, determine the sizes in base pairs of each "unknown" fragment.

   - Find the migration distance of the unknown fragment on the x-axis. Draw a vertical line from that point until the standard graph line is intersected.
   - From the point of intersection, draw a second line horizontally to the y-axis and determine the approximate size of the fragment in base pairs (refer to Figure 1 for an example).

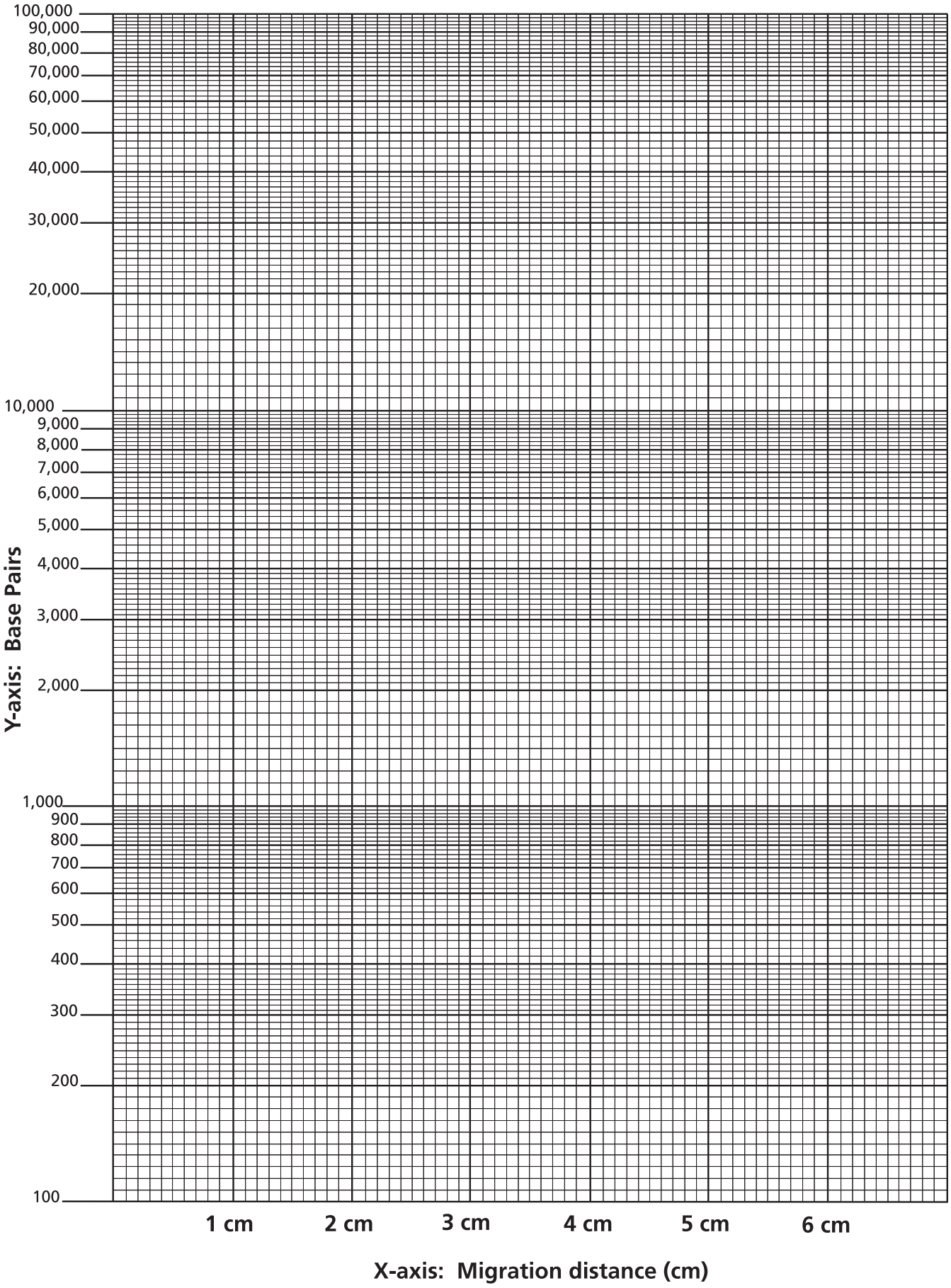
Figure 1



Quick Reference:

Standard DNA fragment sizes - length is expressed in base pairs.

23130	9416	6557
4361	3000	2322
2027	725	570


100,000
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
900
800
700
600
500
400
300
200
100
Y-axis: Base Pairs
1 cm
2 cm
3 cm
4 cm
5 cm
6 cm
X-axis: Migration distance (cm)

Study Questions

1. How should the x and y axes of the semi-log graph paper used in this experiment, be labeled?

2. Determine unknown 1 and unknown 2 DNA fragment sizes in base pairs according to your standard curve, then determine your percentage error.

3. Use the example of the standard curve to determine:

   a. The base pair size if the migration distance is 2.8 centimeters.

   b. The migration distance if the fragment contains 5,500 base pairs.

Notes:

Instructor's Guide

Notes to the Instructor & Pre-Lab Preparations

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in planning and implementing this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances. If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK web site. Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

EDUCATIONAL RESOURCES, NATIONAL CONTENT AND SKILL STANDARDS

By performing this experiment, students will learn to load samples and run agarose gel electrophoresis. Experiment analysis will provide students the means to transform an abstract concept into a concrete explanation.

EDVOTEK Ready-to-Load Electrophoresis Experiments are easy to perform and are designed for maximum success in the classroom setting. However, even the most experienced students and teachers occasionally encounter experimental problems or difficulties. EDVOTEK web site resources provide suggestions and valuable hints for conducting electrophoresis, as well as answers to frequently asked electrophoresis questions.

Laboratory Extensions and Supplemental Activities

Laboratory extensions are easy to perform using EDVOTEK experiment kits. For example, a DNA sizing determination activity can be performed on any electrophoresis gel result if DNA markers are run in parallel with other DNA samples. For DNA Sizing instructions, and other laboratory extension suggestions, please refer to the EDVOTEK website.

Visit the EDVOTEK web site often for continuously updated information.

Notes to the Instructor & Pre-Lab Preparations

APPROXIMATE TIME REQUIREMENTS

1. Gel preparation:
Whether you choose to prepare the gel(s) in advance or have the students prepare their own, allow approximately 30 minutes for this procedure. Generally, 20 minutes of this time is required for gel solidification.

2. Micropipeting and Gel Loading:
If your students are unfamiliar with using micropipets and sample loading techniques, a micropipeting or practice gel loading activity is suggested prior to conducting the experiment. Two suggested activities are:

   - EDVOTEK Expt. # S-44, Micropipetting Basics, focuses exclusively on using micropipets. Students learn pipeting techniques by preparing and delivering various dye mixtures to a special Pipet Card™.
   - Practice Gel Loading: EDVOTEK Series 100 electrophoresis experiments contain a tube of practice gel loading solution for this purpose. It is highly recommended that a separate agarose gel be cast for practice sample delivery. This activity can require anywhere from 10 minutes to an entire laboratory session, depending upon the skill level of your students.

Table C
Time and Voltage Recommendations

Volts	EDVOTEK Electrophoresis Model M6+ Minimum / Maximum	EDVOTEK Electrophoresis Model M12 & M36 Minimum / Maximum
150	15 / 20 min	25 / 35 min
125	20 / 30 min	35 / 45 min
70	35 / 45 min	60 / 90 min
50	50 / 80 min	95 / 130 min

3. Conducting Electrophoresis:
The approximate time for electrophoresis will vary from approximately 15 minutes to 2 hours. Different models of electrophoresis units will separate DNA at different rates depending upon its design configuration. Generally, the higher the voltage applied the faster the samples migrate. However, maximum voltage should not exceed the indicated recommendations. The Table C example at left shows Time and Voltage recommendations. Refer to Table C in Appendices A or B for specific experiment guidelines.

PREPARING AGAROSE GELS FOR ELECTROPHORESIS

There are several options for preparing agarose gels for the electrophoresis experiments:

1. Individual Gel Casting: Each student lab group can be responsible for casting their own individual gel prior to conducting the experiment.

2. Batch Gel Preparation: A batch of agarose gel can be prepared for sharing by the class. To save time, a larger quantity of UltraSpec-Agarose can be prepared for sharing by the class. See instructions for "Batch Gel Preparation".

3. Preparing Gels in Advance: Gels may be prepared ahead and stored for later use. Solidified gels can be stored under buffer in the refrigerator for up to 2 weeks.

Do not store gels at -20°C. Freezing will destroy the gels.

Notes to the Instructor & Pre-Lab Preparations

USING AGAROSE GELS THAT HAVE BEEN PREPARED IN ADVANCE

If gels have been removed from their trays for storage, they should be "anchored" back to the tray with a few drops of hot, molten agarose before placing the gels into the apparatus for electrophoresis. This will prevent the gel from sliding around in the tray and/or floating around in the electrophoresis chamber.

AGAROSE GEL CONCENTRATION AND VOLUME

Gel concentration is one of many factors which affect the mobility of molecules during electrophoresis. Higher percentage gels are sturdier and easier to handle. However, the mobility of molecules and staining will take longer because of the tighter matrix of the gel. Gel volume varies depending on the size of the casting tray, as well as the type of stain to be used for DNA staining after electrophoresis. Gels which will be stained with InstaStain® Ethidium Bromide require less sample amount (volume) than gels that will be stained with FlashBlue™ or InstaStain® Blue.

This experiment requires a 0.8% gel. It is a common agarose gel concentration for separating dyes or DNA fragments in EDVOTEK experiments.

- Specifications for preparing a 0.8% gel to be stained with FlashBlue™ or InstaStain® Blue can be found in Appendix A.
- Specifications for preparing a 0.8% gel to be stained with InstaStain® Ethidium bromide can be found in Appendix B.

Tables A-1 and A-2 below are examples of tables from Appendix A. The first (left) table shows reagent volumes using concentrated (50x) buffer. The second (right) table shows reagent volumes using diluted (1x) buffer.

⇩ If preparing a 0.8% gel with concentrated (50x) buffer, use Table A.1

⇩ If preparing a 0.8% gel with diluted (1x) buffer, use Table A.2

Table A.1

Individual 0.8%* UltraSpec-Agarose™ Gel

DNA Staining with FlashBlue™ or InstaStain® Blue

Size of Gel (cm)	Amt of Agarose (g)	+	Concentrated Buffer (50x) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
7 x 7	0.23		0.6		29.4		30
7 x 10	0.39		1.0		49.0		50
7 x 14	0.46		1.2		58.8		60

Table A.2

Individual 0.8%* UltraSpec-Agarose™ Gel

DNA Staining with FlashBlue™ or InstaStain® Blue

Size of Gel (cm)	Amt of Agarose (g)	+	Diluted Buffer (1x) (ml)
7 x 7	0.23		30
7 x 10	0.39		50
7 x 14	0.46		60

* 0.77 UltraSpec-Agarose™ gel percentage rounded up to 0.8%

Notes to the Instructor & Pre-Lab Preparations

GEL STAINING AND DESTAINING AFTER ELECTROPHORESIS

DNA stains FlashBlue™ and InstaStain® Blue are included in EDVOTEK standard Series 100 experiments. For Series 100-Q experiments, InstaStain® Ethidium Bromide (InstaStain® EtBr) is included. InstaStain® is a proprietary staining method which saves time and reduces liquid waste. EDVOTEK also offers Protein InstaStain® for staining Protein polyacrylamide gels, which can be purchased separately.

Instructions for DNA staining options are provided in the Appendices section.

Option 1: FlashBlue™ liquid - Appendix E.

This simple and rapid liquid staining and destaining procedure yields excellent visibility of DNA bands in less than 25 minutes (5 minutes staining, 20 minutes destaining).

Option 2: InstaStain® Blue cards, One-step Staining and Destaining- Appendix F.

Agarose gels can be stained and destained in one easy step.

Option 3: InstaStain® Blue cards - Appendix G.

Using InstaStain® Blue cards, staining is completed in approximately 5-10 minutes. DNA bands will become visible after destaining for approximately 20 minutes. Results will become sharper with additional destaining. For the best photographic results, allow the gel to destain for several hours to overnight. This will allow the stained gel to "equilibrate" in the destaining solution, resulting in dark blue DNA bands contrasting against a uniformly light blue background.

Option 4: InstaStain® Ethidium Bromide - Appendix H

Staining with ethidium bromide is very sensitive and can detect as little as 5 to 10 nanograms of DNA with the use of a U.V. transilluminator. Ethidium Bromide is a dye that is commonly used by scientific researchers. It is a listed mutagen and forms a tight complex with DNA by intercalating between the bases within the double helix. The complex strongly fluoresces when exposed to ultraviolet light.

CAUTION: Ethidium Bromide is a listed mutagen. Disposal of the InstaStain® EtBr cards, which contain microgram amounts of ethidium bromide, is minimal compared to the large volume of liquid waste generated by traditional ethidium bromide staining procedures. Disposal of InstaStain® cards and gels should follow institutional guidelines for chemical waste.

Notes to the Instructor & Pre-Lab Preparations

READY-TO-LOAD DNA SAMPLES FOR ELECTROPHORESIS

No heating required before gel loading.

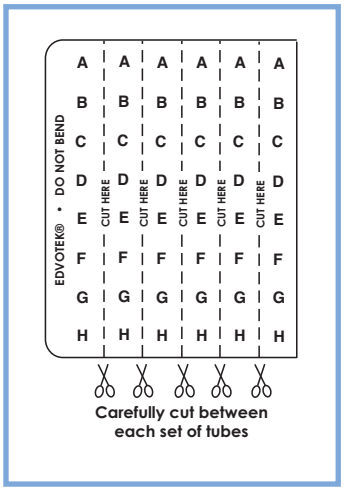
EDVOTEK offers the widest selection of electrophoresis experiments which minimize expensive equipment requirements and save valuable time for integrating important biotechnology concepts in the teaching laboratory. Series 100 experiments feature DNA samples which are predigested with restriction enzymes and are stable at room temperature. DNA samples are ready for immediate delivery onto agarose gels for electrophoretic separation and do not require pre-heating in a waterbath.

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

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

SAMPLES FORMAT: PRE-ALIQUOTED QUICKSTRIP™ CONNECTED TUBES

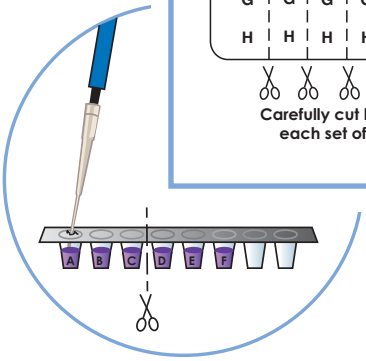
Convenient QuickStrip™ connected tubes contain pre-aliquoted ready-to-load samples. The samples are packaged in a microtiter block of tubes covered with a protective overlay. Follow the instructions below for preparation of QuickStrip™.



1. Use sharp scissors to separate the block of samples into individual strips as shown in the diagram at right.

   The number of samples per set will vary depending on the experiment. Some tubes may be empty.

2. Cut carefully between the rows of samples. Do not cut or puncture the protective overlay directly covering the sample tubes.



3. Next, cut each individual strip between samples C & D.
   - A & D contain Standard Fragments
   - B & E contain Unknown DNA 1
   - C & F contain Unknown DNA 2
   - G & H are intentionally left blank.

4. Each gel will require one strip of samples, either A - C or D - F.

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

Notes to the Instructor & Pre-Lab Preparations

SAMPLES FORMAT: INDIVIDUAL 1.5 ML MICROTEST TUBES

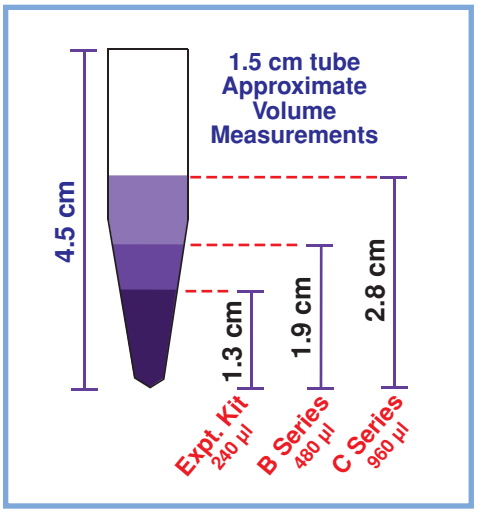
It is recommended that samples packaged in 1.5 ml individual microtest tubes be aliquoted for each gel. DNA Samples packaged in this format are available in three standard quantities:

Standard experiment kit	240 µl	Custom bulk quantities are also available by request.
Bulk B-Series	480 µl	
Bulk C Series	960 µl	

1. Check all sample volumes for possible evaporation. Samples will become more concentrated if evaporation has occurred.

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

   1.3 cm level for Standard experiment kit
   1.9 cm level for the B-Series
   2.8 cm level for the C-Series



3. Mix well by inverting and tapping the tubes several times.

4. After determining that the samples are at their proper total volumes, aliquot each sample into appropriately labeled 0.5 ml or 1.5 ml microtest tubes.

   - For gels to be stained with Flash-Blue™ or InstaStain® Blue:

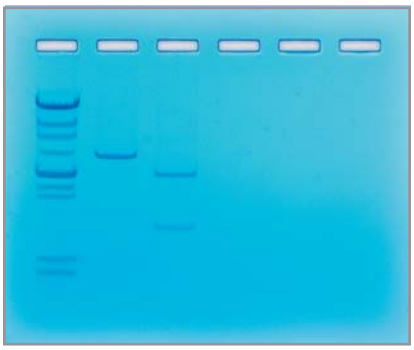
     35-38 µl of each sample

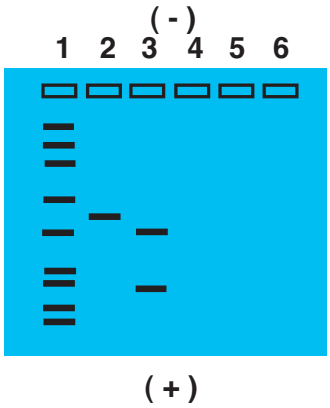
   - For gels to be stained with InstaStain® Ethidium bromide:

     18-20 µl of each sample

5. If students have difficulty retrieving the entire aliquoted volume of sample because some of it clings to the side walls of the tubes, remind students to make sure all of the sample is at the bottom of the tube before gel loading. They should centrifuge the samples tubes, or tap the tubes on the tabletop.

Experiment Results and Analysis





In the idealized schematic, the relative positions of DNA fragments are shown but are not depicted to scale.

Lane	Tube				
1	A or D	Standard DNA Fragments (expressed in approximate base pairs)			
		23130	9416	6557	4361	3000
			2322	2027	725	570
		The 3000 bp and 725 bp fragments have been added to the Hind III fragments to facilitate measurements.			
2	B or E	Unknown DNA 1	4,200 bp	± 650	
3	C or F	Unknown DNA 2	3,000 bp	± 450	
			1,200 bp	± 180	

Study Questions and Answers

1. **How should the x and y axes of the semi-log graph paper used in this experiment, be labeled?**

   The x-axis should be labeled "migration distance" in centimeters; the y-axis should be labeled "base pairs" (1-10,000)

2. **Determine unknown 1 and unknown 2 DNA fragment sizes in base pairs according to your standard curve, then determine your percentage error.**

   Answers will vary. Compare and average values derived by various students in the class.

3. **Use the example of the standard curve to determine:**

   a. **The base pair size if the migration distance is 2.8 centimeters.**

      If the migration distance is 2.8 cm, then the base pair size is 2500 base pairs.

   b. **The migration distance if the fragment contains 5,500 base pairs.**

      If the fragment contains 5,500 base pairs, then the migration distance is 1.7 cm.

Appendices

A 0.8 % Agarose Gel Electrophoresis Reference Tables
For DNA Staining with FlashBlue™ or InstaStain® Blue

B 0.8% Agarose Gel Electrophoresis Reference Tables
For DNA Staining with InstaStain® Ethidium Bromide

C Quantity Preparations for Agarose Gel Electrophoresis

D Agarose Gel Preparation Step by Step Guidelines

E Staining and Visualization of DNA
FlashBlue™ liquid

F Staining and Visualization of DNA
InstaStain® Blue One-step Staining and destaining

G Staining and Visualization of DNA
InstaStain® Blue Cards

H Staining and Visualization of DNA
InstaStain® Ethidium Bromide Cards

Appendix **A**

0.8% Agarose Gel Electrophoresis Reference Tables
(DNA Staining with FlashBlue™ or InstaStain® Blue)

If preparing a 0.8% gel with concentrated (50x) buffer, use Table A.1

If preparing a 0.8% gel with diluted (1x) buffer, use Table A.2

Table A.1 Individual 0.8%* UltraSpec-Agarose™ Gel
DNA Staining with FlashBlue™ or InstaStain® Blue

Size of Gel (cm)	Amt of Agarose (g)	+ Concentrated Buffer (50x) (ml)	+ Distilled Water (ml)	= Total Volume (ml)
7 x 7	0.23	0.6	29.4	30
7 x 10	0.39	1.0	49.0	50
7 x 14	0.46	1.2	58.8	60

Table A.2 Individual 0.8%* UltraSpec-Agarose™ Gel
DNA Staining with FlashBlue™ or InstaStain® Blue

Size of Gel (cm)	Amt of Agarose (g)	+ Diluted Buffer (1x) (ml)
7 x 7	0.23	30
7 x 10	0.39	50
7 x 14	0.46	60

* 0.77 UltraSpec-Agarose™ gel percentage rounded up to 0.8%

Table B Electrophoresis (Chamber) Buffer

EDVOTEK Model #	Total Volume Required (ml)	Dilution: 50x Conc. Buffer (ml)	+ Distilled Water (ml)
M6+	300	6	294
M12	400	8	392
M36	1000	20	980

For DNA analysis, the recommended electrophoresis buffer is Tris-acetate-EDTA, pH 7.8. The formula for diluting EDVOTEK (50x) concentrated buffer is one volume of buffer concentrate to every 49 volumes of distilled or deionized water. Prepare buffer as required for your electrophoresis unit.

Time and Voltage recommendations for EDVOTEK equipment are outlined in Table C.1 for 0.8% agarose gels. The time for electrophoresis will vary from approximately 15 minutes to 2 hours depending upon various factors. Conduct the electrophoresis for the length of time determined by your instructor.

Table C.1 Time and Voltage Guidelines (0.8% Gel)

	EDVOTEK Electrophoresis Model M6+	EDVOTEK Electrophoresis Model M12 & M36
Volts	Minimum / Maximum	Minimum / Maximum
150	15 / 20 min	25 / 35 min
125	20 / 30 min	35 / 45 min
70	35 / 45 min	60 / 90 min
50	50 / 80 min	95 / 130 min

0.8% Agarose Gel Electrophoresis Reference Tables
(DNA Staining with InstaStain® Ethidium Bromide)

Appendix **B**

If preparing a 0.8% gel with concentrated (50x) buffer, use Table A.3

If preparing a 0.8% gel with diluted (1x) buffer, use Table A.4

Table A.3 Individual 0.8%* UltraSpec-Agarose™ Gel
DNA Staining with InstaStain® Ethidium Bromide

Size of Gel (cm)	Amt of Agarose (g)	+	Concentrated Buffer (50x) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
7 x 7	0.15		0.4		19.6		20
7 x 10	0.23		0.6		29.4		30
7 x 14	0.31		0.8		39.2		40

* 0.77 UltraSpec-Agarose™ gel percentage rounded up to 0.8%

Table A.4 Individual 0.8%* UltraSpec-Agarose™ Gel
DNA Staining with InstaStain® Ethidium Bromide

Size of Gel (cm)	Amt of Agarose (g)	+	Diluted Buffer (1x) (ml)
7 x 7	0.15		20
7 x 10	0.23		30
7 x 14	0.31		40

For DNA analysis, the recommended electrophoresis buffer is Tris-acetate-EDTA, pH 7.8. The formula for diluting EDVOTEK (50x) concentrated buffer is one volume of buffer concentrate to every 49 volumes of distilled or deionized water. Prepare buffer as required for your electrophoresis unit.

Table B Electrophoresis (Chamber) Buffer

EDVOTEK Model #	Total Volume Required (ml)	Dilution: 50x Conc. Buffer (ml)	+	Dilution: Distilled Water (ml)
M6+	300	6		294
M12	400	8		392
M36	1000	20		980

Table C.1 Time and Voltage Guidelines
(0.8% Gel)

	EDVOTEK Electrophoresis Model M6+	EDVOTEK Electrophoresis Model M12 & M36
Volts	Minimum / Maximum	Minimum / Maximum
150	15 / 20 min	25 / 35 min
125	20 / 30 min	35 / 45 min
70	35 / 45 min	60 / 90 min
50	50 / 80 min	95 / 130 min

Time and Voltage recommendations for EDVOTEK equipment are outlined in Table C.1 for 0.8% agarose gels. The time for electrophoresis will vary from approximately 15 minutes to 2 hours depending upon various factors. Conduct the electrophoresis for the length of time determined by your instructor.

Appendix C

Quantity Preparations for Agarose Gel Electrophoresis

To save time, the electrophoresis buffer and agarose gel solution can be prepared in larger quantities for sharing by the class. Unused diluted buffer can be used at a later time and solidified agarose gel solution can be remelted.

Table D

Bulk Preparation of Electrophoresis Buffer

Concentrated Buffer (50x) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
60		2,940		3000 (3 L)

Bulk Electrophoresis Buffer

Quantity (bulk) preparation for 3 liters of 1x electrophoresis buffer is outlined in Table D.

Batch Agarose Gels (0.8%)

For quantity (batch) preparation of 0.8% agarose gels, see Table E.1.

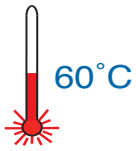
Table E.1

Batch Preparation of 0.8% UltraSpec-Agarose™

Amt of Agarose (g)	+	Concentrated Buffer (50X) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
3.0		7.5		382.5		390

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

1. Use a 500 ml flask to prepare the diluted gel buffer
2. Pour 3.0 grams of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60°C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.



6. Dispense the required volume of cooled agarose solution for casting each gel. The volume required is dependent upon the size of the gel bed and DNA staining method which will be used. Refer to Appendix A or B for guidelines.
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Then proceed with preparing the gel for electrophoresis.

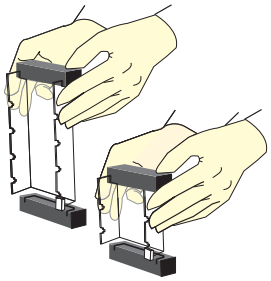
Agarose Gel Preparation - Step by Step Guidelines

Appendix
D

Preparing the Gel bed

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

EDVOTEK electrophoresis units include 7 x 7 cm or 7 x 14 cm gel casting trays.



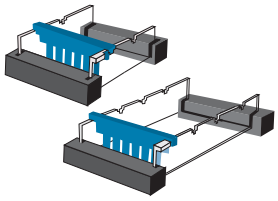
A. Using Rubber dams:

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

B. Taping with labeling or masking tape:

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

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



If gel trays and rubber end caps are new, they may be initially somewhat difficult to assemble. Here is a helpful hint:

Place one of the black end caps with the wide "u" shaped slot facing up on the lab bench.

Push one of the corners of the gel tray into one of the ends of the black cap. Press down on the tray at an angle, working from one end to the other until the end of the tray completely fits into the black cap. Repeat the process with the other end of the gel tray and the other black end cap.

Casting Agarose Gels

3. Use a 250 ml flask or beaker to prepare the gel solution.

4. Refer to the appropriate Reference Table (i.e. 0.8%, 1.0% or 2.0%) for agarose gel preparation. Add the specified amount of agarose powder and buffer. Swirl the mixture to disperse clumps of agarose powder.

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

6. Heat the mixture to dissolve the agarose powder.

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

A. Microwave method:

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

B. Hot plate method:

- Cover the flask with aluminum foil to minimize evaporation.
- Heat the mixture to boiling over a burner with occasional swirling. Boil until all the agarose is completely dissolved.

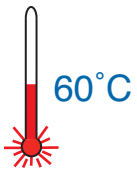
Continue heating until the final solution appears clear (like water) without any undissolved particles. Check the solution carefully. If you see "crystal" particles, the agarose is not completely dissolved.

Appendix
D

Agarose Gel Preparation
Step by Step Guidelines, continued

7. Cool the agarose solution to 60°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 marked in step 5.

DO NOT POUR BOILING HOT AGAROSE INTO THE GEL BED.

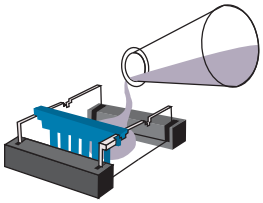


Hot agarose solution may irreversibly warp the bed.

After the gel is cooled to 60°C:

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

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

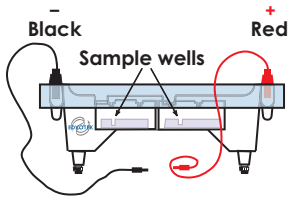


9. Place the bed on a level surface and pour the cooled agarose solution into the bed.

10. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes.

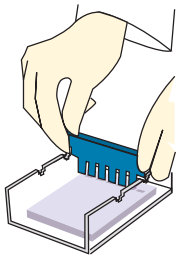
Preparing the gel for electrophoresis

11. After the gel is completely solidified, carefully and slowly remove the rubber dams or tape from the gel bed. Be especially careful not to damage or tear the gel wells when removing the rubber dams. A thin plastic knife, spatula or pipet tip can be inserted between the gel and the dams to break possible surface tension.



During electrophoresis, the DNA samples migrate through the agarose gel towards the positive electrode.

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 appropriate amount of diluted (1x) electrophoresis buffer (refer to Table B on the Appendix page provided by your instructor).

15. Make sure that the gel is completely submerged under buffer before proceeding to loading the samples and conducting electrophoresis.

5 minute Staining

FlashBlue™

Staining and Visualization of DNA FlashBlue™ Liquid Stain

Appendix E

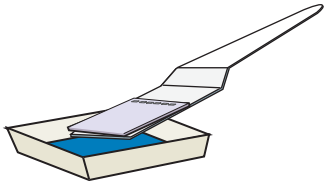
Wear Gloves and Goggles

Preparation of FlashBlue™ Stain from Concentrated Solution

- Dilute 10 ml of 10x FlashBlue™ with 90 ml of distilled or deionized water in a flask. Mix well.
- Cover the flask and store it at room temperature until ready for gel staining.

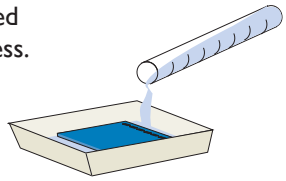
Do not stain gel(s) in the electrophoresis apparatus.

Staining and Destaining

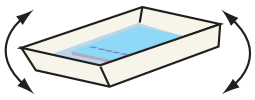


1. Remove the agarose gel from its bed and and completely submerse the gel in a small, clean weighboat or lid from pipet tip rack containing 75 ml of 1x FlashBlue™ stain. Add additional stain if needed to completely submerge the gel.
2. Stain the gel for 5 minutes.

   Note: Staining the gel for longer than 5 minutes will necessitate an extended destaining time. Frequent changes of distilled water will expedite the process.

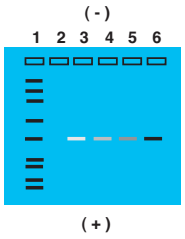


3. Transfer the gel to another small tray and fill it with 250 - 300 ml of distilled water.
4. Gently agitate the tray every few minutes. Alternatively, place it on a shaking platform.



5. Destain the gel for 20 minutes.

   Dark blue bands will become visible against a light blue background. Additional destaining may yield optimal results.



6. Carefully remove the gel from the destaining liquid and examine the gel on a Visible Light Gel Visualization System. To optimize visibility, use the amber filter provided with EDVOTEK equipment.

Storage and Disposal of FlashBlue™ Stain and Gel

- Gels stained with FlashBlue™ 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. FlashBlue™ stain and destaining solutions can be disposed down the drain.

Appendix
F

One-Step Staining and Destaining with InstaStain® Blue

Agarose gels can be stained and destained in one easy step with InstaStain® Blue cards. This one-step method can be completed in approximately 3 hours, or can be left overnight.

Wear Gloves and Goggles

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

1. Remove the 7 x 7 cm agarose gel from its bed and completely submerse the gel in a small, clean tray containing 75 ml of distilled or deionized water, or used electrophoresis buffer. The agarose gel should be completely covered with liquid.

   Examples of small trays include large weigh boats, or small plastic food containers

2. Wearing gloves, gently float a 7 x 7 cm card of InstaStain® Blue with the stain side (blue) facing the liquid.

   Note: If staining a 7 x 14 cm gel, use two InstaStain® Blue cards.

3. Let the gel soak undisturbed in the liquid for approximately 3 hours. The gel can be left in the liquid overnight (cover with plastic wrap to prevent evaporation).

4. After staining and destaining, the gel is ready for visualization and photography.

Storage and Disposal of InstaStain® Blue Cards and Gels

- Stained gels 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!

- Used InstaStain® cards and destained gels can be discarded in solid waste disposal.

- Destaining solutions can be disposed down the drain.

Appendix G

Staining and Visualization of DNA
Instastain® Blue Cards

Wear Gloves and Goggles

1. After electrophoresis, place the agarose gel on a flat surface covered with plastic wrap.
2. Wearing gloves, place the blue dye side of the InstaStain® Blue card(s) on the gel.
3. Firmly run your fingers several times over the entire surface of the InstaStain® card to establish good contact between the InstaStain® card and the gel.
4. To ensure continuous contact between the gel and the InstaStain® card, place a gel casting tray and weight, such as a small empty beaker, on top of the InstaStain® card.
5. Allow the InstaStain® Blue to sit on the gel for 5 to 10 minutes.
6. After staining, remove the InstaStain® card.

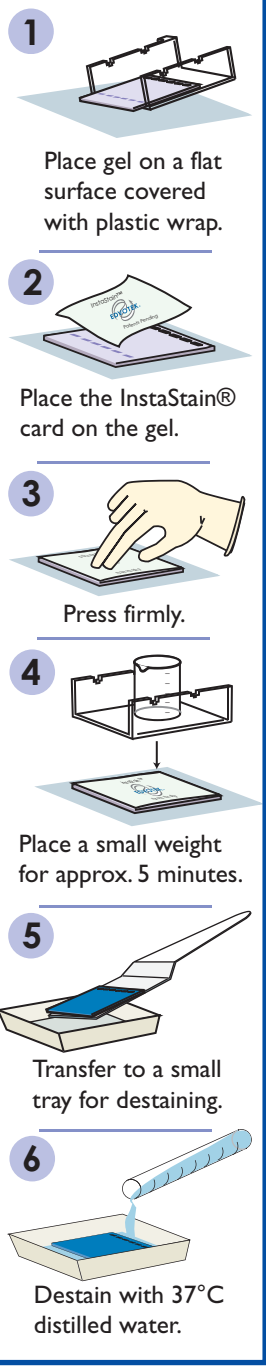
   If the color of the gel appears very light, wet the gel surface with buffer or distilled water and place the InstaStain® card on the gel for an additional 5 minutes.

Destaining and Visualization of DNA

7. Transfer the gel to a large weigh boat or small plastic container.
8. Destain with approximately 100 ml of distilled water to cover the gel.
9. Repeat destaining by changing the distilled water as needed.

   Larger DNA bands will initially be visible as dark blue bands against a lighter blue background. When the gel is completely destained, larger DNA bands will become sharper and smaller bands will be visible. With additional destaining, the entire background will become uniformly light blue. Destaining time may vary between 20 - 90 minutes.

10. 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.
11. If the gel is too light and bands are difficult to see, repeat the staining and destaining procedures.



InstaStain is a registered trademark of EDVOTEK, Inc. Patents Pending.

Appendix
G

Staining and Visualization of DNA
Instastain® Blue Cards
continued

Destaining Notes for InstaStain® Blue

- Use of warmed distilled water at 37°C will accelerate destaining. Destaining will take longer with room temperature water.
- DO NOT EXCEED 37°C ! Warmer temperatures will soften the gel and may cause it to break.
- The volume of distilled water for destaining depends upon the size of the tray. Use the smallest tray available that will accommodate the gel. The gel should be completely submerged during destaining.
- Do not exceed 3 changes of water for destaining. Excessive destaining will cause the bands to be very light.

Storage and Disposal of InstaStain® Blue Cards and Gels

- Stained gels 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!
- Used InstaStain® cards and destained gels can be discarded in solid waste disposal.
- Destaining solutions can be disposed down the drain.

Appendix
H

Staining and Visualization of DNA InstaStain® Ethidium Bromide Cards

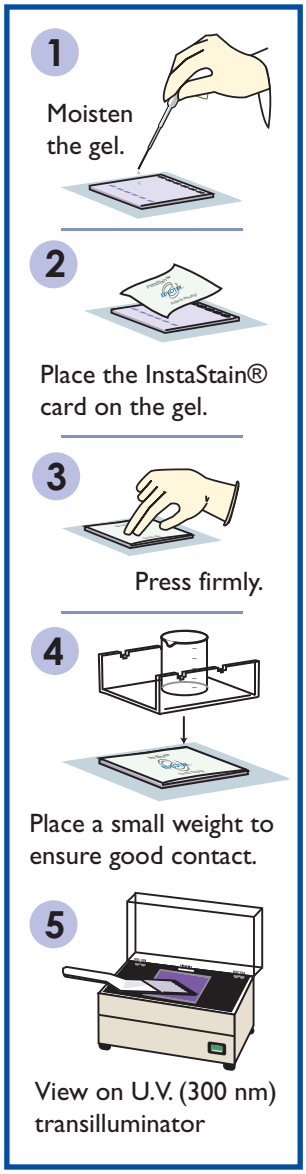
Wear Gloves and Goggles

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

1. After electrophoresis, place the gel on a piece of plastic wrap on a flat surface. Moisten the gel with a few drops of electrophoresis buffer.
2. Wearing gloves, remove the clear plastic protective sheet, and place the unprinted side of the InstaStain® EtBr card(s) on the gel.
3. Firmly run your fingers over the entire surface of the InstaStain® EtBr. Do this several times.
4. Place the gel casting tray and a small empty beaker on top to ensure that the InstaStain® card maintains direct contact with the gel surface.

   Allow the InstaStain® EtBr card to stain the gel for 3-5 minutes.
5. After 10-15 minutes, remove the InstaStain® EtBr card. Transfer the gel to a ultraviolet (300 nm) transilluminator for viewing. Be sure to wear UV protective goggles.

Caution: Ethidium Bromide is a listed mutagen.



Disposal of InstaStain

Disposal of InstaStain® cards and gels should follow institutional guidelines for chemical waste.

Additional Notes About Staining

- If bands appear faint, or if you are not using EDVOTEK UltraSpec-Agarose™, gels may take longer to stain with InstaStain® EtBr. Repeat staining and increase the staining time an additional 10-15 minutes.
- DNA markers should be visible after staining even if other DNA samples are faint or absent. If markers are not visible, troubleshoot for problems with the electrophoretic separation.

Material Safety Data Sheets

Full-size (8.5 x 11") pdf copy of MSDS is available at www. edvotek.com or by request.

EDVOTEK®

Material Safety Data Sheet

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

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

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

Section I

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

Address (Number, Street, City, State, Zip Code): **1121 5th Street NW Washington DC 20001**

Emergency Telephone Number: **202-370-1500**

Telephone Number for information: **202-370-1500**

Date Prepared: 11-16-11

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

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

Section III - Physical/Chemical Characteristics

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

Solubility in Water: Soluble

Appearance and Odor: Blue liquid, no odor

Section IV - Physical/Chemical Characteristics

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

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

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

Unusual Fire and Explosion Hazards: Unknown

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable	X	None

Incompatibility: None

Hazardous Decomposition or Byproducts: Sulfur oxides, and bromides

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

Section VI - Health Hazard Data

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

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

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

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

Medical Conditions Generally Aggravated by Exposure: None reported

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

Section VII - Precautions for Safe Handling and Use

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

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

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

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation	Local Exhaust	Yes	Special	None	
	Mechanical (General)	Yes	Other	None	
Protective Gloves	Yes		Eye Protection	Splash proof goggles	

Other Protective Clothing or Equipment: None required

Work/Hygienic Practices: Avoid eye and skin contact

EDVOTEK®

Material Safety Data Sheet

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

IDENTITY (As Used on Label and List): **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): **1121 5th Street NW Washington DC 20001**

Emergency Telephone Number: **202-370-1500**

Telephone Number for information: **202-370-1500**

Date Prepared: 11-16-11

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	Unstable		Conditions to Avoid
	Stable	X	None

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): None

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

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

Medical Conditions Generally Aggravated by Exposure: None

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

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

Section VII - Precautions for Safe Handling and Use

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

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

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

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation	Local Exhaust	Yes	Special	None	
	Mechanical (General)	Yes	Other	None	
Protective Gloves	Yes		Eye Protection	_Safety goggles	

Other Protective Clothing or Equipment: None

Work/Hygienic Practices: None

EDVOTEK®

Material Safety Data Sheet

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

IDENTITY (As Used on Label and List): **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): **1121 5th Street NW Washington DC 20001**

Emergency Telephone Number: **202-370-1500**

Telephone Number for information: **202-370-1500**

Date Prepared: 11-16-11

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 0 = 1) 2	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Insoluble - cold

Appearance and Odor: White powder, no odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

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

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

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

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable	X	None

Incompatibility: No data available

Hazardous Decomposition or Byproducts:

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

Section VI - Health Hazard Data

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

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

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: No data available

Medical Conditions Generally Aggravated by Exposure: No data available

Emergency First Aid Procedures: Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

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

Waste Disposal Method: Normal solid waste disposal

Precautions to be Taken in Handling and Storing: None

Other Precautions: None

Section VIII - Control Measures

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

Ventilation	Local Exhaust		Special		
	Mechanical	Gen. dilution ventilation	Other		
Protective Gloves	Yes		Eye Protection	Splash proof goggles	

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

Work/Hygienic Practices: None

EDVOTEK®

Material Safety Data Sheet

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

IDENTITY (As Used on Label and List): InstaStain® Blue, FlashBlue™

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): **1121 5th Street NW Washington DC 20001**

Emergency Telephone Number: **202-370-1500**

Telephone Number for information: **202-370-1500**

Date Prepared: 11-16-11

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, 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	Unstable		Conditions to Avoid: None
	Stable	X	

Incompatibility: Strong oxidizing agents

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

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): 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:

EDVOTEK®

Material Safety Data Sheet

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

IDENTITY (As Used on Label and List): InstaStain® Ethidium Bromide

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. 1121 5th Street NW Washington DC 20001**

Emergency Telephone Number: **202-370-1500**

Telephone Number for information: **202-370-1500**

Date Prepared: 11-16-11

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)
Ethidium Bromide			Data not available	
(2,7-Diamino-10-Ethyl-9-Phenylphenanthridinium Bromide)				
CAS# 139-33-3				

Section III - Physical/Chemical Characteristics

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

Solubility in Water: Soluble

Appearance and Odor: Chemical bound to paper, 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, carbon dioxide, dry chemical powder, alcohol or polymer foam

Special Fire Fighting Procedures: Wear protective clothing and SCBA to prevent contact with skin & eyes

Unusual Fire and Explosion Hazards: Emits toxic fumes

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid: None
	Stable	X	

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide, nitrogen oxides, hydrogen bromide gas

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): Chronic: May alter genetic material
Acute: Material irritating to mucous membranes, upper respiratory tract, eyes, skin

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

Signs and Symptoms of Exposure: Irritation to mucous membranes and upper respiratory tract

Medical Conditions Generally Aggravated by Exposure: No data

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: Wear SCBA, rubber boots, rubber gloves

Waste Disposal Method: Mix material with combustible solvent and burn in a chemical incinerator equipped afterburner and scrubber

Precautions to be Taken in Handling and Storing: Use in chemical fume hood with proper protective lab gear.

Other Precautions: Mutagen

Section VIII - Control Measures

Respiratory Protection (Specify Type): SCBA

Ventilation	Local Exhaust	Yes	Special	Chem. fume hood	
	Mechanical (General)	No	Other	None	

Protective Gloves	Rubber	Eye Protection	Chem. safety goggles

Other Protective Clothing or Equipment: Rubber boots

Work/Hygienic Practices: Use in chemical fume hood with proper protective lab gear.

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