EDVOTEK®

The Biotechnology Education Company ®

Revised and Updated

EDVO-Kit #

302

Purification of the Restriction Enzyme *Eco* RI

Storage: See Page 3 for specific storage instructions

EXPERIMENT OBJECTIVE:

In this experiment, students will purify a restriction endonuclease, test its enzyme activity, and visualize the test results by agarose gel electrophoresis.

This experiment is designed for DNA staining with InstaStain® Ethidium Bromide.

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EVT 2010-08-17K

Table of Contents

Components & Requirements

This experiment is designed for DNA staining with InstaStain® Ethidium Bromide.

Experiment Components

This experiment is designed for 5 laboratory groups.

	Components	Storage
A	*E. coli* RY (*Eco* RI) Extract (lyophilized)	Refrigerator
B	DEAE-Cellulose	Room temperature
C	10x Equilibration Buffer	Freezer
D	50% Glycerol	Freezer
E	KCl	Room temperature
F	*Eco* RI Reaction Buffer	Freezer
G	Qualified Water	Freezer
H	Lambda DNA	Freezer
I	Lambda/*Eco* RI Marker	Freezer
J	*Eco* RI Dilution Buffer	Freezer

Reagents & Supplies

- UltraSpec-Agarose™ powder
- Concentrated electrophoresis buffer
- 10x Gel Loading Solution
- Chromatography columns
- InstaStain® Ethidium Bromide

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.

STORAGE OF PERISHABLES

This experiment includes perishable components which were sent on wet ice. Store these components at -20°C (-4°F). Please note what type of freezer you have and store components accordingly.

Frost-free Freezer

Most refrigerator/freezers in homes are frost free. This means the freezer goes through warming cycles to eliminate frost (defrost cycle). If using this type of freezer, keep the enzymes in the foam chest (with the ice brick) in which they were sent. This will help maintain the enzymes at -20°C when the freezer goes through the defrost cycle.

Non Frost-free Freezer

These older model freezers, which are still sold but are harder to find, do not go through warming cycles. Therefore, ice will build up on freezer walls over time. If using this type of freezer, check to make sure that it maintains temperature at -20°C.

EDVO-Kit # 302

Purification of the Restriction Enzyme *Eco* RI

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips
- Balance
- Water bath
- Ring stand and clamps
- 13 x 100 mm glass test tubes
- Assorted laboratory glassware
- Permanent markers and tape
- 1.5 ml microtest tubes
- Microwave, hot plate or burner
- Pipet pump
- 250 ml flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Distilled or deionized water
- UV Transilluminator

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Purification of the Restriction Enzyme *Eco* RI

Sequence-specific, or Type II, endonucleases are commonly known as restriction enzymes. In contrast with nonspecific endonucleases, these enzymes generate reproducible fragments from specific DNAs. They cleave double-stranded DNA by hydrolyzing two phosphodiester bonds (one per strand) within defined nucleotide sequences. Over 3,000 enzymes have been discovered since the first report by H.O. Smith and collaborators. These enzymes are extracted from a variety of bacterial strains.

Restriction Enzyme	Recognition Site
Bam HI *Bacillus amyloliquefaciens* H	5'-GGATCC-3' 3'-CCTAGG-5'
Bgl I *Escherichia coli* RY13	5'-GCCNNNNNGGC-3' 3'-CGGNNNNNCCG-5'
Eco RI *Bacillus globigii*	5'-GAATTC-3' 3'-CTTAAG-5'
Hae III *Haemophilus aegyptius*	5'-GGCC-3' 3'-CCGG-5'
Hind III *Haemophilus influenzae* R4	5'-AAGCTT-3' 3'-TTCGAA-5'

Figure 1: Examples of Restriction Enzymes and their recognition sites

The name of a restriction enzyme is derived from the genus and species of bacterium from which it is isolated. The first letter of the genus name and first two letters of the species are combined to form the enzyme name. This is followed by a strain designation if applicable. In many instances, a bacterial strain contains more than one restriction endonuclease. When this occurs, each enzyme is assigned a Roman numeral. For example, *Bam* HI was the first enzyme activity reported from *Bacillus amyloliquefaciens* strain H.

Most restriction enzymes are composed of two polypoeptides of equal subunits with molecular weights of 20,000-25,000 or single polypeptides with molecular weights of 30,000-35,000. Enzyme activities can be differentiated from each other by their characteristic digestion patterns of small viral DNAs. The DNA from bacteriophage lambda is the most widely used substrate for screening restriction enzymes. Because it is often difficult to determine a characteristic pattern from a lambda digest, smaller DNAs, such as the replicative form of bacteriophage ØX174 and SV40 DNA are also used as substrates. The resulting DNA restriction enzyme digests are displayed on agarose gels and visualized by staining with ethidium bromide.

A given recognition sequence in DNA can often be cleaved by more than one restriction enzyme. The term "isoschizomers" describes a group of restriction enzymes that recognize the same sequence in DNA. The sequences recognized by these enzymes are for the most part centrosymmetric "palindromic" sequences that are usually hexamers, pentamers, or tetramers. Several Type II restriction enzymes recognize DNA at a specific site and hydrolyze phosphodiester bonds at a defined distance from that site. An example of this group of enzymes is *Bgl* I, which recognizes a sequence containing two groups of specified residues separated by completely unspecified residues - GCCNNNNNGGC; it therefore generates DNA fragments with variable end groups.

Purification of the Restriction Enzyme *Eco* RI

Background Information

There is considerable diversity in the fragment termini produced in cleavage by Type II endonucleases that recognize and cleave within the same sequence. In some cases, the 5' extension may be as short as two nucleotides or as long as five. Points of cleavage on each strand may be opposite each other; this results in blunt (square ends). Several restriction endonucleases produce 3' extensions of two to four nucleotides. However, all Type II endonucleases produce fragments with a 5'-terminal phosphate and a 3'-terminal hydroxyl residue (Figure 1).

Enzymes in the Type II restriction enzyme family are amenable to purification by chromatographic procedures. Ion exchangers at nearly neutral pH are used as separation matrices after extracts have been freed of cellular nucleic acids. At this stage of purification, short-term assays often make it possible to visualize enzyme fractions that contain restriction enzymes. A variety of enzymes have been fractionated with affinity chromatography. This method takes advantage of biospecific interactions not offered by conventional fractionation methods. The advantages of affinity chromatography are speed of purification and often protection against denaturation during fractionation.

Effects of Reaction Conditions on Restriction Enzymes

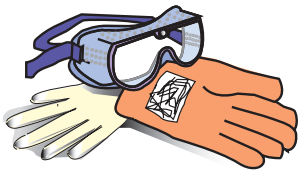
Several reports have described apparent changes in specificity of restriction endonucleases in association with altered reaction environments. Conditions that alter specificity have included changes in ionic concentration, pH of the reaction buffer, and the amounts of glycerol in the storage and the reaction mixture. For example, when lambda DNA is incubated with *Eco* RI or *Bam* HI in the presence of glycerol at various concentrations, a progressive change in the DNA digestion pattern is observed.

A change in recognition specificity of enzymes include *Bam* HI and *Eco* RI activity. The second activity is designated as ".1" (as *Bam* HI.1). A similar activity is displayed by *Eco* RI. Increasing the pH of the reaction from 7.0 to 9.0 in the absence of monovalent cations stimulates alternate activities. Decreases in the ionic strength have a similar effect.

Experiment Overview and General Instructions

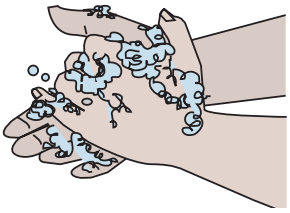
EXPERIMENT OBJECTIVE:

In this experiment, students will purify a restriction endonuclease, test its enzyme activity, and visualize the test results 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.



6. This experiment utilizes InstaStain® Ethidium Bromide for staining and visualization of DNA after gel electrophoresis. Always wear gloves when handling InstaStain® cards. Alhough there is only a very small amount of Ethidium bromide on InstaStain® EtBr cards, it is a listed mutagen. Wear UV-resistant safety goggles when working with ultraviolet light since it can cause irreparable damage to the eyes. Exposure to skin should also be avoided.

Partial Purification of *Eco* RI

The Experiment

Note

Loading the column and subsequent elution will be done at room temperature. The elution buffers and the fractions collected should be stored on ice as they elute from the column.

PACKING AND EQUILIBRATING THE COLUMN

1. Vertically mount the column on a ring stand. Make sure it is straight.
2. Slide the cap onto the spout at the bottom of the column.
3. Mix the DEAE-Cellulose (ion-exchanger matrix) thoroughly by swirling or gently stirring.
4. Carefully pipet the mixed DEAE-Cellulose into the column by letting it stream down the inside walls of the column.

   If the flow is stopped by an air pocket, stop adding the DEAE-Cellulose and firmly tap the column until the air is removed and the exchanger flows down. Continue adding the exchanger.
5. Place an empty beaker under the column to collect wash material.
6. Remove the cap from the bottom of the column and allow the matrix to pack into the column.
7. Wash the packed column with 25 ml of Eq (1x equilibration buffer).

Do not allow the column to dry.

Partial Purification of *Eco* RI

COLLECTING COLUMN FRACTIONS

1. Label eight pieces of tape 2-9 with a permanent lab marker and adhere to 13 x 100 mm test tubes. The chart to the left indicates which tubes to use for the different fractions.

2. Add 3 ml of distilled water to a test tube and use this as a reference guide for collecting the eluted fractions.

3. Slowly load the column with 1 ml of *E. coli* RY extract. Allow the extract to completely enter the column.

4. Slowly add 6 ml of Eq to the column to remove protein that is in the flow through. Collect two fractions (3 ml each) into the tubes labeled 2 and 3 and store on ice.

5. Sequentially elute the column with the following buffers. In each case, collect 3 ml fractions into the appropriate tubes and store fractions on **ice** immediately upon collection. Do not let the column go dry.

   - 6 ml of 0.1 M KCl. Collect two fractions, 3 ml each, into tubes 4 and 5. Store on ice.
   - 6 ml of 0.2 M KCl. Collect two 3 ml fractions into tubes 6 and 7. Store on ice.
   - 6 ml of 0.5 M KCl. Collect two 3 ml fractions into tubes 8 and 9. Store on ice.

Table 1: Key for Identifying Fractions

Tube	Fraction
2	(no salt)
3	(no salt)
4	0.1 M KCl
5	0.1 M KCl
6	0.2 M KCl
7	0.2 M KCl
8	0.5 M KCl
9	0.5 M KCl

OPTIONAL STOPPING POINT

If time does not permit you to continue with *Eco* RI Activity analysis, you may freeze the fractions at -20°C and perform the assays at a later time. Thaw the fractions at room temperature and immediately place on ice. Continue with the analysis of *Eco* RI Activity.

The Experiment

Analysis of Eco RI Activity (First Assay)

Lambda DNA will be incubated with the fractions collected and the samples will be electrophoresed in an agarose gel to determine the peak activity of *Eco* RI endonuclease. Lambda DNA cut with *Eco* RI yields a characteristic and recognizable fragmentation pattern.

Important Note

Reagents listed in Table 2 will be used for two assays. Label your set of reagent tubes with your initials or group number and store reagents in the refrigerator between assays.

1. With a permanent marker, label 9 microtest tubes 1-9. Put your initials and group number on each tube.
2. Each group will assay *Eco* RI using 2 µl, 4 µl, 6 µl, 8 µl, or 10 µl as assigned by your instructor. In Table 3 on the next page, the "x" equals your assigned volume for analysis.
3. Use an automatic micropipet to add (40-x µl) of Qualified water to each of the 9 tubes
4. Use an automatic micropipet to dispense 5 µl of the *Eco* RI Rxn Buffer and 5 µl of Lambda DNA to each of the 9 tubes.
5. Use a clean pipet tip for each fraction and add 2 µl, 4 µl, 6 µl, 8 µl, or 10 µl as assigned, from each fraction to the appropriate tube.

Table 2: Reagents for the Incubation of *Eco* RI with Lambda DNA

Water (G)	1 ml	on ice
Eco RI Rxn Buffer (F)	100 µl	on ice
Lambda DNA (H)	100 µl	on ice
10x Gel Load	100 µl	
Marker	45 µl	

6. Cap the tubes tightly and tap on the lab bench to collect samples at the bottom of the tubes or quick spin balanced tubes in a microcentrifuge.
7. Mix the samples and incubate in a 37°C waterbath for 15 minutes.
8. Make sure your set of reagent tubes are labeled with your initials or group number and store in the refrigerator for later use in the second assay.
9. After the 15 minute incubation is complete, add 5 µl of 10x gel loading solution to each tube to stop the reactions.

   This prepares the *Eco* RI digestion products for separation by agarose gel electrophoresis.

Analysis of Eco RI Activity (First Assay)

Sequence for Restriction Enzyme Reactions

Rxn Tube	Qualified Water (µl)	*Eco* RI Reaction Buffer (µl)	Lambda DNA (µl)	Fraction	Reaction Volume (µl)	37°C Incubation (minutes)	10x Gel Load (µl)
1	40	5	5	None	50	15	5
2	(40 - x)	5	5	x µl tube 2 (no salt)	50	15	5
3	(40 - x)	5	5	x µl tube 3 (no salt)	50	15	5
4	(40 - x)	5	5	x µl tube 4 (0.1 M KCl)	50	15	5
5	(40 - x)	5	5	x µl tube 5 (0.1 M KCl)	50	15	5
6	(40 - x)	5	5	x µl tube 6 (0.2 M KCl)	50	15	5
7	(40 - x)	5	5	x µl tube 7 (0.2 M KCl)	50	15	5
8	(40 - x)	5	5	x µl tube 8 (0.5 M KCl)	50	15	5
9	(40 - x)	5	5	x µl tube 9 (0.5 M KCl)	50	15	5

* Volumes of *Eco* RI in fractions should be varied among different groups within the range of 2 to 5 µl, with 1 µl increments. Water in the assay should be adjusted accordingly.

** To be added after incubation at 37°C.

OPTIONAL STOPPING POINT

If time does not permit you to continue with agarose gel electrophoresis at this time, you may freeze the fractions at -20°C and perform the electrophoresis at a later date. Thaw the fractions at room temperature and heat the samples at 65°C before loading the gel.

The Experiment

Analysis of *Eco* RI Activity (First Assay)

If you are unfamiliar with agarose gel preparation and electrophoresis, detailed instructions and helpful resources are available at **www.edvotek.com**

AGAROSE GEL REQUIREMENTS FOR THE FIRST ASSAY

- Recommended gel size: 7 x 14 cm
- Number of sample wells required: 10
- Agarose gel concentration: 0.8%

PREPARING THE AGAROSE GEL

1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.
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.
3. To a 250 ml flask or beaker, add agarose powder and buffer as indicated in the Reference Tables (Appendix A) provided by your instructor. Swirl the mixture to disperse clumps of agarose powder.
4. With a marking pen, indicate the level of the solution volume on the outside of the flask.

Important Note

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.

5. Heat the mixture using a microwave oven or burner to dissolve the agarose powder.
6. 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 4.

After the gel is cooled to 60°C:

7. Place the bed on a level surface and pour the cooled agarose solution into the bed.
8. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes.
9. After the gel is solidified, be careful not to damage or tear the wells while removing the rubber dams or tape and comb(s) from the gel bed.
10. Place the gel (on its bed) into the electrophoresis chamber, properly oriented, centered and level on the platform.
11. Fill the electrophoresis apparatus chamber with the appropriate amount of diluted (1x) electrophoresis buffer (refer to Table B on the instruction sheet from the Appendix provided by your instructor).

Analysis of *Eco* RI Activity (First Assay)

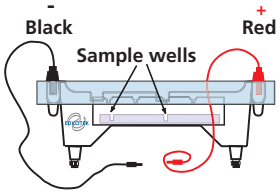
LOADING THE SAMPLES

This experiment is designed for staining with InstaStain® Ethidium Bromide. The amount of sample that should be loaded is 18-20 µl. Make sure the gel is completely submerged under buffer before loading the samples and conducting electrophoresis.

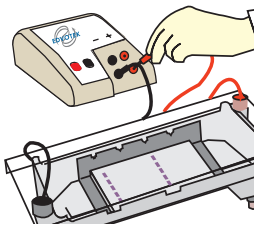
Lane	Tube	
1	Marker	Lambda Eco RI Marker
2	1	Uncut Lambda DNA
3	2	Lambda + 2 (no salt)
4	3	Lambda + 3 (no salt)
5	4	Lambda + 4 (0.1 M KCl)
6	5	Lambda + 5 (0.1 M KCl)
7	6	Lambda + 6 (0.2 M KCl)
8	7	Lambda + 7 (0.2 M KCl)
9	8	Lambda + 8 (0.5 M KCl)
10	9	Lambda + 9 (0.5 M KCl)

Reminder:

Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.



RUNNING THE GEL



Electrophoresis can be completed in 15-20 minutes under optimal conditions. For Time and Voltage recommendations, refer to Table C (from Appendix A or B).

1. After the DNA samples are loaded, properly orient the cover and carefully snap it onto the electrode terminals.
2. Insert the plugs of the black and red wires into the corresponding inputs of the power source.
3. Set the power source at the required voltage and conduct electrophoresis for the length of time determined by your instructor.
4. Check to see that current is flowing properly - you should see bubbles forming on the two platinum electrodes.
5. After the electrophoresis is completed, disconnect the power and remove the gel from the bed for staining.

STAINING AND VISUALIZATION OF DNA

After electrophoresis, agarose gels require staining to visualize the separated DNA samples. Your instructor will provide instructions for DNA staining with InstaStain® Ethidium Bromide.

The Experiment

Quantification of *Eco* RI Activity (Second Assay)

Units of enzyme activity are defined by convention. A restriction enzyme unit is defined as the amount of enzyme activity that will digest 1 µg of lambda DNA at 37°C within one hour under the defined assay conditions. To determine the total units of *Eco* RI purified in this experiment, re-assay pooled enzyme fractions at various enzyme dilutions to determine the minimum amount of enzyme that yields complete digestion of 1 µg of lambda DNA.

Table 4: Dilution of Pooled *Eco* RI

Pooled Enzyme (µl)	Dilution Buffer (µl)	Total Volume (µl)	Dilution Factor
10	0	10	0
5	5	10	1:2
10	20	30	1:3
10	30	40	1:4
10	90	100	1:10
5	95	100	1:20

Store all fractions on ice.

Label fractions according to dilution factors. 10 µl of each dilution will be used as shown in Table 5.

1. Pool the enzyme fractions that have *Eco* RI activity as judged by the first assay. If a fraction has only a trace of activity, do not pool it since it will dilute the enzyme which may result in activity loss.
2. Measure and record the volume of pooled *Eco* RI fractions.
3. Gently mix the pooled fraction to get a representative sample for assaying.
4. Dilute the pooled *Eco* RI enzyme fraction with *Eco* RI Dilution Buffer, using the dilution factors indicated in Table 4.
5. Prepare each *Eco* RI dilution (from Table 4) for incubation as outlined in Table 5. Use the remaining reagents from the first assay that were stored in the refrigerator.
6. After completing the incubations as outlined in Table 5, add 10x Gel Loading Solution to each tube to stop the reactions.
7. Separate the *Eco* RI digestion products by agarose gel electrophoresis.

Table 5: Assay to Determine Total Units of *Eco* RI

Rxn Tube	Qualified Water (µl)	*Eco* RI Reaction Buffer (µl)	Lambda DNA (µl)	*Eco* RI Dilution (from Table 4)	Reaction Volume (µl)	37°C Incubation (minutes)	10x Gel Load* (µl)
1	40	5	5	None	50	30	5
2	30	5	5	10 µl of 0	50	30	5
3	30	5	5	10 µl of 1:2	50	30	5
4	30	5	5	10 µl of 1:3	50	30	5
5	30	5	5	10 µl of 1:4	50	30	5
6	30	5	5	10 µl of 1:10	50	30	5
7	30	5	5	10 µl of 1:20	50	30	5

*To be added after 37°C incubation

Quantification of *Eco* RI Activity (Second Assay)

AGAROSE GEL REQUIREMENTS FOR THE SECOND ASSAY

- Recommended gel size: 7 x 14 cm
- Number of sample wells required: 8
- Agarose gel concentration: 0.8%

1. Prepare a 0.8% agarose gel for the second assay according to instructions previously described.

2. Load 20 µl of each DNA sample in the following manner:

Lane	Tube	
1	Marker	Lambda *Eco* RI Marker
2	1	Uncut Lambda DNA
3	2	Lambda + Undiluted *Eco* RI
4	3	Lambda + 1:2 Dilution
5	4	Lambda + 1:3 Dilution
6	5	Lambda + 1:4 Dilution
7	6	Lambda + 1:10 Dilution
8	7	Lambda + 1:20 Dilution

3. After the samples are loaded, conduct electrophoresis and stain the gel with InstaStain® Ethidium Bromide for visualization.

3. Examine the gel or take a photograph to determine which lane gives complete digestion determined as follows:

- No undigested or partially digested lambda DNA is visible.
- All the DNA digestion products (5 bands) are visible.

The Experiment

Activity Determination in Units

Restriction enzyme unit = amount of enzyme activity that will digest 1 µg of lambda DNA at 37°C within one hour·

DETERMINATION OF TOTAL ACTIVITY

Total units (units) is the amount of enzyme activity recovered from the preparation. It does not indicate the level of enzyme purity.

$$\text{Total Activity (units)} = \frac{\text{Pooled volume (µl)}}{\text{Volume used for assay (µl)}} \times \text{Dilution factor}$$

Example for Determining Total units.

Pooled volume is 9 ml = 9000 µl
Eco RI volume for assay = 10 µl
Dilution factor = 4

$$\frac{9000\ \mu l}{10\ \mu l} \times 4 = 3600 \text{ units}$$

for a 30 minute digestion

Conversion for a 1 hour digestion assay:

Total Activity units = 3600 units X 2 = 7200

SPECIFIC ACTIVITY DETERMINATION (OPTIONAL)

Specific activity is defined as the number of enzyme units per mg of total protein in the enzyme fraction. The less total protein the *Eco* RI fraction contains, the higher is its specific activity.

- For this experiment we have equated 1.0 absorbance unit at A_{280}. In 9 ml, the amount of protein is 0.2 mg/ml X 9 ml = 1.8 ml.

$$\text{Specific Activity} = \frac{\text{Total units}}{\text{mg of protein}}$$

Example for Determining Specific Activity

Total units: 7200 units for the total volume of 9 ml
Total mg. of protein - 1.8 mg

$$\text{Specific Activity} = \frac{7200 \text{ units}}{1.8 \text{ mg}} = 4{,}000 \text{ units/mg}$$

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. What is the recognition site for *Eco* RI?
2. How is *E. coli* host DNA protected against action of the *Eco* RI endonuclease?
3. How many *Eco* RI sites are there in lambda DNA?
4. What is the difference between total activity versus specific activity?

Instructor's Guide

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines 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. In addition, 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

Electrophoresis Hints, Help and Frequently Asked Questions

EDVOTEK Experiments are designed for maximum success in the classroom setting. However, even the most experienced students and teachers occasionally encounter experimental problems or difficulties. The EDVOTEK web site provides several suggestions and reminders for conducting electrophoresis, as well as answers to frequently asked electrophoresis questions.

Notes to the Instructor:

The Experiment

MICROPIPETTING BASICS AND PRACTICE GEL LOADING

Accurate pipeting is critical for maximizing successful experiment results. EDVOTEK Series 300 experiments are designed for students who have had previous experience with agarose gel electrophoresis and micropipeting techniques. If your students are unfamiliar with using micropipets, EDVOTEK highly recommends that students perform Experiment # S-44, Micropipetting Basics, or other Series 100 or 200 electrophoresis experiment prior to conducting this advanced level experiment.

APPROXIMATE TIME REQUIREMENTS

- **Pre-lab preparations**
  Pre-lab preparations and dispensing of biologicals and reagents take approximately 1-2 hours.

- **Restriction Enzyme Digestion**
  The approximate time required for students to perform the restriction enzyme digestion and prepare samples for electrophoresis is 50-75 minutes. Extending the restriction enzyme digest incubation time to 60 minutes will help ensure complete cleavage of DNA.

- **Agarose Gel preparation**
  Whether you choose to prepare the gel(s) in advance or have the students prepare their own, allow approximately 30-40 minutes for this procedure. Generally, 20 minutes of this time is required for gel solidification. See section "Options for Preparing Agarose Gels" below.

Table C
Time and Voltage Recommendations

Volts	EDVOTEK Electrophoresis Model M6+ Minimum / Maximum	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

- **Conducting Electrophoresis**
  The approximate time for electrophoresis will vary from 15 minutes to 2 hours. Generally, the higher the voltage applied, the faster the samples migrate. However, depending upon the apparatus configuration and the distance between the two electrodes, individual electrophoresis units will separate DNA at different rates. Follow manufacturer's recommendations. Time and Voltage recommendations for EDVOTEK equipment are outlined in Table C.

Notes to the Instructor:

OPTIONS FOR PREPARING AGAROSE GELS

This experiment is designed for DNA staining after electrophoresis with InstaStain® Ethidium Bromide. There are several options for preparing agarose gels for the experiment.

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

2. 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.

   Gels that have been removed from their trays for storage, 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 gels from sliding around in the trays and the chambers.

3. 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".

GEL CONCENTRATION AND VOLUME

The gel concentration required is 0.8%. Prepare 7 x 14 cm gels according to Table A.1 or A.2 in Appendix A.

Notes to the Instructor:

GEL STAINING AND DESTAINING AFTER ELECTROPHORESIS

This experiment features InstaStain® Ethidium Bromide for gel staining after electrophoresis. It is a proprietary staining method which saves time and reduces liquid waste. DNA staining with InstaStain® Methylene Blue is not recommended because it will not yield optimal results.

Instastain® Ethidium Bromide

- InstaStain® Ethidium Bromide Appendix C

Optimal visualization of DNA fragments on gels is obtained by staining with InstaStain® Ethidium Bromide (InstaStain® EtBr) cards.

Caution: Ethidium Bromide is a listed mutagen. Disposal of the InstaStain® EtBr cards, which contain only a few micrograms 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.

PHOTODOCUMENTATION OF DNA (OPTIONAL)

There are many different photodocumentation systems available, including digital systems that are interfaced directly with computers. Specific instructions will vary depending upon the type of photodocumentation system you are using.

Pre-Lab Preparations

The 10x equilibration buffer used to hydrate the DEAE-Cellulose contains potassium phosphate, pH 7.4, EDTA, and β-mercaptoethanol.

PARTIAL PURIFICATION OF *ECO* RI
(Packing the Column and Collecting Fractions)

DEAE-Cellulose Matrix

1. Hydrate the ion-exchanger, DEAE-Cellulose (B) in 35 ml of 10x equilibration buffer (C).
2. Stir occasionally for a minimum of 30 minutes.
3. Aliquot 6 ml for each of the five groups.

Buffers

4. Prepare 500 ml of 1x equilibration buffer (Eq) in a 600 ml flask or beaker. To prepare, add the following and stir thoroughly:

350 ml	Distilled water
50 ml	10x Equilibration buffer (C)
100 ml	50% glycerol (D)

Use this prepared Eq buffer to make buffers in step 5.

Quick Reference

Summary of Reagent Preparations

DEAE-Cellulose (Matrix, B)	6 ml	on ice
Eq Buffer (1x equil buffer, diluted C)	35 ml	on ice
0.1 M KCl	6 ml	on ice
0.2 M KCl	6 ml	on ice
0.5 M KCl	6 ml	on ice
E. coli RY extract (A)	1 ml	on ice

Each group requires:

- DEAE-Cellulose
- Eq Buffer
- 0.1 M KCl
- 0.2 M KCl
- 0.5 M KCl
- *E. coli* RY extract
- 1 chromatography column
- 1 ring stand with clamp
- 9 test tubes (13 x 100 mm)
- 10 microtest tubes
- Automatic micropipet & tips
- 5 ml pipets and pipet pumps

5. To make Eq + KCl Buffers, mix the following:

	Eq Buffer (1x)	KCl
0.1 M KCl	100 ml	0.75 g
0.2 M KCl	100 ml	1.5 g
0.5 M KCl	100 ml	3.75 g

E. coli Cell Extract Containing Eco RI Restriction Enzyme

6. Re-hydrate the sample by adding 0.5 ml of distilled or deionized water to tube component A and let sit for 5 minutes.
7. Mix vigorously by vortexing and transfer the entire contents to a 50 ml conical tube. Rinse tube A six times - each time with 1 ml of 1x Equilibration buffer (diluted component C) and add the rinse material to the 50 ml conical tube. Mix the tube well.
8. Label 5 tubes "*E. coli* RY extract". Aliquot 1 ml of the re-hydrated extract for each of the student groups. Store extracts on ice.

Instructor's Guide

Instructor's Guide

Pre-Lab Preparations

ANALYSIS AND QUANTIFICATION OF *ECO* RI ACTIVITY (First and Second Assays)

Incubation of Fractions with Lambda DNA

Important: Students should be reminded that the reagents they receive are for two assays.

1. Label 5 tubes "water" and dispense 1 ml Qualified Water (G) into the tubes. Store on ice.
2. Label 5 tubes "*Eco* RI Rxn Buffer" and dispense 100 µl of *Eco* RI Reaction Buffer (F) into the tubes. Store on ice.

Quick Reference

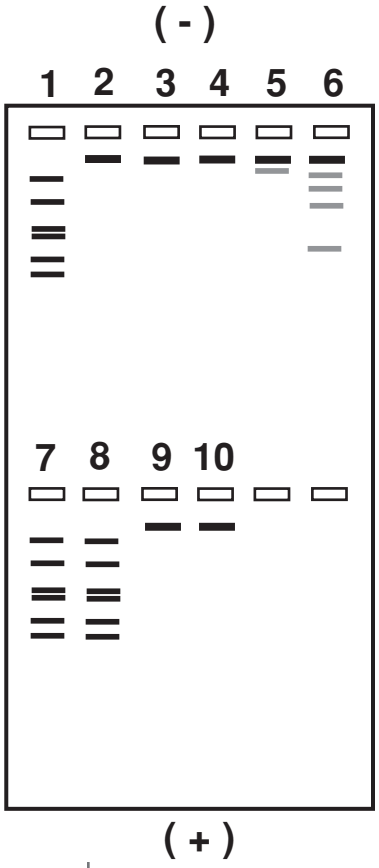
Summary of Reagent Preparations

Reagents for First & Second Assays		
Water (G)	1 ml	on ice
Eco RI Rxn Buffer (F)	100 µl	on ice
Lambda DNA (H)	100 µl	on ice
10x Gel Load	100 µl	
Marker	45 µl	
Additional Reagent for Second Assay		
Lambda DNA (H)	250 µl	on ice

3. Label 5 tubes "Lambda DNA" and dispense 100 µl of Lambda DNA (H) into the tubes. Store on ice.
4. Label 5 tubes "10x Gel Load" and dispense 100 µl 10x Gel Loading Solution into the tubes.
5. Label 5 tubes "Marker" and dispense 45 µl Lambda/*Eco* RI Marker (I) into the tubes.
6. Label 5 tubes "*Eco* RI Diln Buffer" and dispense 250 µl of *Eco* RI Dilution Buffer (J) into the tubes. Store on ice.
7. Have a 37°C waterbath ready for *Eco* RI activity analysis.

Experiment Results and Analysis

PARTIAL PURIFICATION OF *ECO* RI (FIRST ASSAY)



In the idealized schematic, the relative positions of DNA fragments are shown but are not depicted to scale. The schematic depicts an idealized gel result for identifying column fractions with *Eco* RI activity.

Lane	Tube	
1	Marker	Lambda *Eco* RI Markers
2	1	Uncut Lambda DNA
3	2	Lambda + 2 (no salt)
4	3	Lambda + 3 (no salt)
5	4	Lambda + 4 (0.1 M KCl)
6	5	Lambda + 5 (0.1 M KCl)
7	6	Lambda + 6 (0.2 M KCl)
8	7	Lambda + 7 (0.2 M KCl)
9	8	Lambda + 8 (0.5 M KCl)
10	9	Lambda + 9 (0.5 M KCl)

* Results may vary between different groups and from the schematic depicted to the left. Some bands may be faint and thus difficult to see. You may also see extra bands due to partial digestion of the DNA. The amount of activity in the flow through may also vary.

Results of the second assay will show varying results depending upon the amount of purified enzyme activity.

Instructor's Guide

Please refer to the kit insert for the Answers to Study Questions

Appendices

Appendices

A 0.8% Agarose Gel Preparation For DNA Staining with InstaStain® Ethidium Bromide

B 0.8% Quantity Preparations for Agarose Gel Electrophoresis

C Staining and Visualization of DNA with InstaStain® Ethidium Bromide Cards

Material Safety Data Sheets

EDVO-Kit # 302

Appendix A

0.8% Agarose Gel Preparation Reference Tables for DNA Staining with InstaStain® Ethidium Bromide

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

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

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

Size of Gel (cm)	Amt of Agarose (g)	+ Concentrated Buffer (50X) (ml)	+ Distilled Water (ml)	= **Total Volume (ml)**
7 x 7	0.2	0.5	24.5	**25**
7 x 14	0.4	1.0	49.0	**50**

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

Table A.2 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	0.2	25
7 x 14	0.4	50

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)	+ Distilled Water (ml)
M6+	300	6	294
M12	400	8	392
M36 (blue)	500	10	490
M36 (clear)	1000	20	980

Table C Time and Voltage Recommendations

Volts	EDVOTEK Electrophoresis Model: M6+ Minimum / Maximum	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

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

Appendix B

EDVO-Kit # 302

0.8% Agarose Gel Electrophoresis Reference Tables
Quantity Preparations

To save time, 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 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)

Table E

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

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

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.

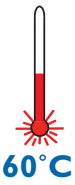
BULK ELECTROPHORESIS BUFFER

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. 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.
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.

EDVO-Kit # 302

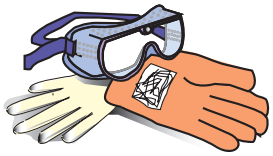
Appendix C

Staining and Visualization of DNA

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

INSTASTAIN® ETHIDIUM BROMIDE CARDS

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 on the gel.
3. Firmly run your fingers over the entire surface of the InstaStain® EtBr. Do this several times.



Wear gloves and safety goggles

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.

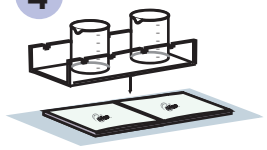
   Allow the InstaStain® EtBr card to stain the gel for 10-15 minutes.

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.

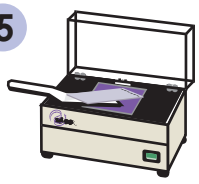
1 Moisten the gel.

2 Place the InstaStain® card on the gel.

3 Press firmly.



4 Place a small weight to ensure good contact.



5 View on U.V. (300 nm) transilluminator

Visit our web site for an animated demonstration of InstaStain® EtBr.

www.edvotek.com

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.
- Gels stained alternatively with InstaStain Methylene Blue or liquid methylene blue may fade with time. Re-stain the gel to visualize the DNA bands.
- DNA 200 bp markers should be visible after staining even if the amplified DNA samples are faint or absent. If markers are not visible, troubleshoot for problems with the electrophoretic separation.

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: 10/05/06

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): No data	Flammable Limits	LEL: N.D	UEL: N.D.

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

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

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid: None
	Stable	X	

Incompatibility: No data available

Hazardous Decomposition or Byproducts:

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): 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

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: 10/05/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

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

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity ($H_2 0$ = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

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

Appearance and Odor: Clear, liquid, slight vinegar odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

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

Extinguishing Media: Use extinguishing media appropriate for surrounding fire.

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

Unusual Fire and Explosion Hazards: None identified

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid: None
	Stable	X	

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide

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): 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): Enzyme Reaction/ Dilution 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: 11/07/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

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

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity ($H_2 0$ = 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): No data	Flammable Limits	LEL: No data	UEL: 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	Unstable		Conditions to Avoid: 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	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): Toxicity has not been quantified. Sensitivity reactions (allergic) may occur by skin penetration including anaphylactic shock.

Carcinogenicity: None NTP? 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 Sheets

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

EDVO-Kit # 302

EDVO-Kit # 302

Material Safety Data Sheets

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

Material Safety Data Sheet

EDVOTEK®

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: InstaStain, Inc.
P.O. Box 1232
West Bethesda, MD 20827

Emergency Telephone Number: (301) 251-5990

Telephone Number for information: (301) 251-5990

Date Prepared: 10/05/06

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
	Stable	X	None

Incompatibility: Strong oxidizing agents

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

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): 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.

Material Safety Data Sheet

EDVOTEK®

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): 50% Glycerol

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: 12/01/06

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)
CAS # 56-81-5				

Section III - Physical/Chemical Characteristics

Boiling Point	182°C	Specific Gravity (H_2O = 1)	1.26
Vapor Pressure (mm Hg.)	20°C <1MM	Melting Point	20°C
Vapor Density (AIR = 1)	3.1	Evaporation Rate (Butyl Acetate = 1)	N/A

Solubility in Water:

Appearance and Odor: Viscous colorless liquid

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL

Extinguishing Media: Water spray, CO_2, Dry chemical powder or appropriate foam

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

Unusual Fire and Explosion Hazards: Emits toxic fumes under fire conditions.

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable	X	

Incompatibility: Strong oxidizing agents, strong bases, protect from moisture

Hazardous Decomposition or Byproducts: CO, CO_2

Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): May Cause skin irritation-prolonged exposure can cause headache, nausea and vomiting

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure:

Medical Conditions Generally Aggravated by Exposure:

Emergency First Aid Procedures: Flush with copious amounts of H20 for at least 15 minutes . Remove to fresh air & remove contaminated clothing

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Absorb on sand or vermiculate and place in closed container for disposal. Ventilate area and wash spill site

Waste Disposal Method: Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with afterburner

Precautions to be Taken in Handling and Storing: Don't breathe vapor, avoid contact with eyes, skin and clothing wash thoroughly after handling. Keep tightly closed.

Other Precautions:

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation X	Local Exhaust		Special		
	Mechanical (General)	Mechanical exhaust	Other		
Protective Gloves	Chemical resistant		Eye Protection	Safety goggles	

Other Protective Clothing or Equipment:

Work/Hygienic Practices: Wash thoroughly after handling

Material Safety Data Sheet

EDVOTEK®

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: 10/10/06

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	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	Unstable		Conditions to Avoid
	Stable	X	None

Incompatibility: None known

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: 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.

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)	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.
Potassium Chloride	

Section I

Manufacturer's Name	Emergency Telephone Number
EDVOTEK, Inc.	**(301) 251-5990**
Address (Number, Street, City, State, Zip Code)	Telephone Number for information
14676 Rothgeb Drive Rockville, MD 20850	**(301) 251-5990**
	Date Prepared 12/01/06
	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)
None	N/A	N/A	N/A	N/A

Section III - Physical/Chemical Characteristics

Boiling Point	1500°C	Specific Gravity (H_2O = 1)	1.984
Vapor Pressure (mm Hg.)	N/A	Melting Point	N/A
Vapor Density (AIR = 1)	N/A	Evaporation Rate (Butyl Acetate = 1)	N/A
Solubility in Water	Soluble		
Appearance and Odor	White crystalline powder, odorless		

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	N/A	Flammable Limits	LEL N/A	UEL N/A
Extinguishing Media	Dry chemical, carbon dioxide, water spray or foam.			
Special Fire Fighting Procedures	Wear SCBA and protective clothing			
Unusual Fire and Explosion Hazards	None known			

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid: Violent reaction w/ BrF3, (H_2SO_4 + $KMNO_4$
	Stable	X	
Incompatibility	BrF3		
Hazardous Decomposition or Byproducts	N/A		
Hazardous Polymerization	May Occur		Conditions to Avoid: N/A
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry:	Inhalation? Yes	Skin? Yes	Ingestion? Yes	
Health Hazards (Acute and Chronic)	Presents no serious health effects in normal industrial uses. However, dust may cause irritation of eyes,			
Carcinogenicity:	NTP?	IARC Monographs? No data	OSHA Regulation?	
Signs and Symptoms of Exposure	Irritation of eyes, skin and mucous membranes, vomiting, weakness and circulatory problems			
Medical Conditions Generally Aggravated by Exposure	Unknown			
Emergency First Aid Procedures	Eyes and skin: Flush with copious amounts of water for 15 min. Inhalation: move to fresh air Ingestion: get medical attention. Never give anything by mouth to an unconscious person			

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled
Sweep up material and place into suitable waste container. Flush spill area with water.

Waste Disposal Method
Dispose of material in accordance with state, local and federal regulations.

Precautions to be Taken in Handling and Storing
Store in tightly sealed container. Protect from moisture.

Other Precautions
Avoid skin contact and avoid breathing dust.

Section VIII - Control Measures

Respiratory Protection (Specify Type)	NIOSH/MSHA approved respirator			
Ventilation	Local Exhaust	Recommended	Special	N/A
	Mechanical (General)	Yes	Other	N/A
Protective Gloves	PE or rubber gloves	Eye Protection	Safety glasses or goggles	
Other Protective Clothing or Equipment	Lab coat			
Work/Hygienic Practices	Wash thoroughly after use.			