

The Biotechnology Education Company ®

EDVO-Kit

101

Principles and Practice of Agarose Gel Electrophoresis

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

The objective of this experiment is to develop a basic understanding of electrophoretic theory, and to gain "hands-on" familiarity with the procedures involved in horizontal gel electrophoresis to separate different molecules.

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Table of Contents

	Page
Experiment Components	3
Experiment Requirements	3
Background Information	4
Experiment Procedures Experiment Overview and General Instructions	5
Agarose Gel Electrophoresis	7
Study Questions	8
Instructor's Guidelines	
Notes to the Instructor and Pre-Lab Preparations	9
Experiment Results and Analysis	13
Study Questions and Answers	14
Appendices	15
Material Safety Data Sheets	20

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.

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101

Experiment

Experiment Components

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

Dye samples do not require heating prior to gel loading.

READY-TO-LOAD™ DYE SAMPLES FOR ELECTROPHORESIS

- A Orange
- B Purple
- C Red
- D Blue 1
- E Dye Mixture
- F Blue Dye Mixture (Blue 1 + Blue 2)

REAGENTS & SUPPLIES

- UltraSpec-Agarose™ powder
- Concentrated electrophoresis buffer
- 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
- Flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Visualization system (white light)
- Distilled or deionized water



101

Principles and Practice of Agarose Gel Electrophoresis

Experiment

Background Information

Agarose gel electrophoresis is widely used to separate molecules based upon charge, size and shape. It is particularly useful in separating charged biomolecules such as DNA, RNA and proteins.

Agarose gel electrophoresis possesses great resolving power, yet is relatively simple and straightforward to perform. The gel is made by dissolving agarose powder in boiling buffer solution. The solution is then cooled to approximately 55°C and poured into a gel tray where it solidifies. The tray is submerged in a buffer-filled electrophoresis apparatus which contains electrodes.

Samples are prepared for electrophoresis by mixing them with components that will give the mixture density, such as glycerol or sucrose. This makes the samples denser than the electrophoresis buffer. These samples can then be loaded with a micropipet or transfer pipet into wells that were created in the gel by a template during casting. The dense samples sink through the buffer and remain in the wells.

A direct current power supply is connected to the electrophoresis apparatus and current is applied. Charged molecules in the sample enter the gel through the walls of the wells. Molecules having a net negative charge migrate towards the positive electrode (anode) while net positively charged molecules migrate towards the negative electrode (cathode). Within a range, the higher the applied voltage, the faster the samples migrate. The buffer serves as a conductor of electricity and to control the pH. The pH is important to the charge and stability of biological molecules.

Agarose is a polysaccharide derivative of agar. In this experiment, UltraSpec Agarose™ is used. This material is a mixture of agarose and hydrocolloids which renders the gel to be both clear and resilient. The gel contains microscopic pores which act as a molecular sieve. The sieving properties of the gel influences the rate at which a molecule migrates. Smaller molecules move through the pores faster than larger ones. Molecules can have the same molecular weight and charge but different shapes. Molecules having a more compact shape (a sphere is more compact than a rod) can move faster through the pores.

Factors such as charge, size and shape, together with buffer conditions, gel concentrations and voltage, affects the mobility of molecules in gels. Given two molecules of the same molecular weight and shape, the one with the greater amount of charge will migrate faster. In addition, different molecules can interact with agarose to varying degrees. Molecules that bind more strongly to agarose will migrate more slowly.

In this experiment, several different dye samples will be applied to an agarose gel electrophoresis and their rate and direction of migration will be observed. Dyes A, B, C and D are all negatively charged at neutral pHs. However, these molecules differ with respect to their structure, chemical composition and the amount of charge they carry. Dye F has a net positive charge and therefore will migrate in the opposite direction of the other dyes. This experiment will also demonstrate the ability of agarose gel electrophoresis to separate the mixture of dyes into their individual components by the application of a combination of dyes to the same sample well.



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Experiment Overview and General Instructions

EXPERIMENT OBJECTIVE:

The objective of this experiment is to develop a basic understanding of electrophoretic theory, and to gain "hands-on" familiarity with the procedures involved in agarose gel electrophoresis to separate different molecules.

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.
- 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:

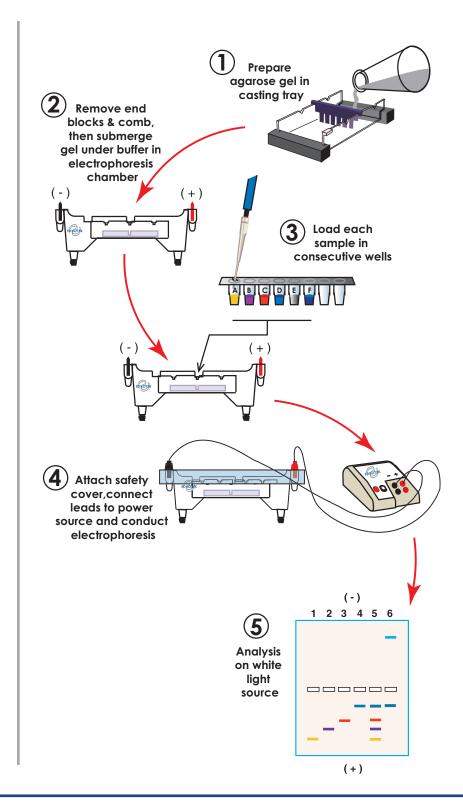
• Record (draw) your observations, or photograph the results.

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

Prepare the Gel

1. Prepare an agarose gel with specifications summarized below.



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

Agarose gel concentration required: 0.8%

Recommended gel size: 7 x 10 cm or 7 x 14 cm

• Number of sample wells required: 6

• Placement of well-former template: Middle set of notches (7 x 10 cm)

Middle set of notches (7 x 14 cm)

Load the Samples

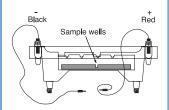
2. Load 35 - 38 µl dye samples in tubes A - F into the wells in consecutive order.

Lane Tube

- Α Orange 1
- В **Purple** 2
- C Red 3
- 4 D Blue 1
- 5 Ε **Dve Mixture**
- Blue Dye Mixture (Blue 1 + Blue 2) F

Reminders:

During electrophoresis, the Dye 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.



Run the Gel

- After dye samples are loaded, connect the apparatus to the direct current (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.
- After electrophoresis is completed, transfer the gel to a white light box for visualization.
- 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 bands.

Note dyes do not require staining - Analyze and document results immediately following gel electrophoresis (dyes will diffuse and will eventually fade from the gel).



101

Principles and Practice of Agarose Gel Electrophoresis

Experiment

Study Questions

- 1. On what basis does agarose gel electrophoresis separate molecules?
- 2. Explain migration according to charge.
- 3. What conclusion can be drawn from the results of sample F?
- 4. Why is glycerol added to the sample solutions before they are loaded into the wells?
- 5. What would happen if distilled water were substituted for buffer in either the chamber solution or the gel solution?



101

Experiment

Instructor's Guide

Notes to the Instructor & Pre-Lab Preparations

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Visit our web site for information about EDVOTEK's complete line of experiments for biotechnology and biology education.

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.

Technical Service
Department

Mon - Fri
9:00 am to 6:00 pm ET

FAX: (301) 340-0582
Web: www.edvotek.com
email: edvotek@aol.com

Please have the following
information ready:

- Experiment number and title
- Kit lot number on box or tube
- Literature version number (in lower right corner)
- Approximate purchase date

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 dye sizing determination activity can be performed on any electrophoresis gel result if dye markers are run in parallel with other dye samples. For dye sizing instructions, please visit our website. For a laboratory extension to this experiment, we suggest Cat. #S-45.

Visit the EDVOTEK web site often for continuously updated information.

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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				Ü
EDVOTEK I			EDVOTEK Electi	ophoresis Model
	Volts		M6+	M12 & M36
			Minimum / Maximum	Minimum / Maximum
	150		15 / 20 min	20 / 30 min
	125		20 / 25 min	30 / 40 min
	70		30 / 40 min	50 / 80 min
	50		45 / 60 min	75 / 120 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 <u>under</u> buffer in the refrigerator for up to 2 weeks.

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



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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 onto the electrophoresis tray 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.

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 can be found in Appendix A.

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

Table A. I						
		Individ	lual 0.8%	* UltraSpec-A	Agarose™	Gel
		of Gel cm)	Amt of Agarose (g)	Concentrated + Buffer (50x) + (ml)	Distilled Water = (ml)	Total Volume (ml)
	7 :	× 7	0.23	0.6	29.4	30
	7 ×	10	0.39	1.0	49.0	50
	7 x	: 14	0.46	1.2	58.8	60

A.2 Individual 0.8%* UltraSpec-Agarose™ Gel							
Si	ze of Gel (cm)	Amt of Agarose + (g)	Diluted Buffer (1x) (ml)				
7 × 7		0.23	30				
7	× 10	0.39	50				
7	× 14	0.46	60				

^{* 0.77} UltraSpec-Agarose $^{\text{TM}}$ gel percentage rounded up to 0.8%





Notes to the Instructor & Pre-Lab Preparations

READY-TO-LOAD 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 dye or DNA samples which are predigested with restriction enzymes and are stable at room temperature. 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 are packaged in one of the following ways:

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

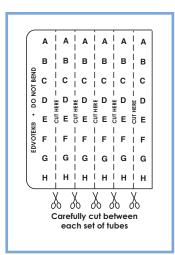
SAMPLES FORMAT: PRE-ALIQUOTED QUICKSTRIP™ CONNECTED TUBES

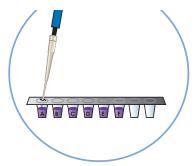
Convenient QuickStrip™ connected sample tubes contain pre-aliquoted ready-to-load samples. The samples are packaged in a microtiter block of tubes covered with a protective overlay. Separate the microtiter block of tubes into strips for a complete set of samples for one gel.

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

Each row of samples (strip) constitutes a complete set of samples for each gel. 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.
- Each gel will require one strip of samples.
- Remind students to tap the tubes before gel loading to ensure that all of the sample is at the bottom of the tube.







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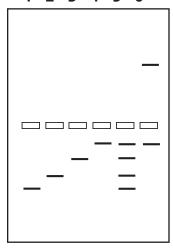
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Experiment Results and Analysis

1 2 3 4 5 6



1 2 3 4 5 6



Lane Tube

- 1 A Orange
- 2 B Purple
- 3 C Red
- 4 D Blue 1
- 5 E Dye Mixture
- 6 F Blue Dye Mixture (Blue 1 + Blue 2)

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





Study Questions and Answers

1. On what basis does agarose gel electrophoresis separate molecules?

Agarose gel electrophoresis separates molecules based on size, charge and shape.

2. Explain migration according to charge.

Molecules having a negative charge migrate toward the positive electrode; positively charged molecules migrate toward the negative electrode.

3. What conclusion can be drawn from the results of sample F?

The color blue has no relationship to charge. Blue 2 has a positive charge; Blue 1 has a negative charge.

4. Why is glycerol added to the sample solutions before they are loaded into the wells?

Glycerol adds density to the samples so they sink through the buffer and into the wells.

5. What would happen if distilled water were substituted for buffer in either the chamber solution or the gel solution?

No ions are contained in distilled water. Ions are required for conductivity of the fluid and therefore, the ability of the molecules to migrate through the gel.



Instructor's Guide

101

Experiment

Appendices

- A 0.8 % Agarose Gel Electrophoresis Reference Tables
- B Quantity Preparations for Agarose Gel Electrophoresis
- C Agarose Gel Preparation Step by Step Guidelines



101

Principles and Practice of Agarose Gel Electrophoresis

Experiment

Appendix

Α

0.8% Agarose Gel Electrophoresis Reference Tables

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

Table A. I	Individ	lual 0.8%	(*	UltraSpec	-A	garose	тм	Gel
Size	of Gel	Amt of	+	Concentrated Buffer (50x) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
7 :	× 7	0.23		0.6		29.4		30
7 ×	10	0.39		1.0		49.0		50
7 ×	: 14	0.46		1.2		58.8		60

^{* 0.77} UltraSpec-Agarose $^{\text{TM}}$ gel percentage rounded up to 0.8%

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

Table 4.2	Individual 0.8%* UltraSpec-Agarose™ Gel				
Si	ze of Gel (cm)	Amt of Agarose + (g)	Diluted Buffer (Ix) (ml)		
7 × 7		0.23	30		
7	× 10	0.39	50		
7	× 14	0.46	60		

г					
	Table B	Elect	Chamber) I	Buffer	
		OVOTEK 1odel #	Total Volume Required (ml)	Dilu 50x Conc. Buffer (ml)	ution H Distilled Water (ml)
		M6+	300	6	294
		MI2	400	8	392
		M36	1000	20	980

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.

	L			
Table Time and Voltage Recommendations				
	EDVOTEK Electrophoresis Model			
Volts		M6+	M12 & M36	
		Minimum / Maximum	Minimum / Maximum	
150		15 / 20 min	20 / 30 min	
125		20 / 25 min	30 / 40 min	
70		30 / 40 min	50 / 80 min	
50		45 / 60 min	75 / I20 min	



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Experiment 101

Quantity Preparations for Agarose Gel Electrophoresis

Appendix **B**

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		ılk Prepa ctrophor	ration of esis Buffer
Concentrated Buffer (50x) - (ml)		Distilled Water (ml)	Total = Volume (ml)
	60	2,940	3000 (3 L)

Table E. I	0.8	Batch Pr 8% UltraS	eparatio pec-Aga	
Amt Agar (g)	ose +	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.

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
- 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.



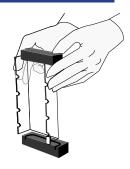
- 6. Dispense the required volume of cooled agarose solution for casting each gel. The volume required is dependent upon the size of the gel bed. Refer to Appendix A for guidelines.
- 7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Then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



Experiment

Principles and Practice of Agarose Gel Electrophoresis

Appendix



Agarose Gel Preparation - Step by Step Guidelines

Preparing the Gel bed

- 1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.
 - A. Using Rubber dams:
 - Place a rubber dam on each end of the bed. Make sure the rubber dam fits firmly in contact with the sides and bottom of the bed.
 - B. Taping with labeling or masking tape:
 - 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.
- Place a well-former template (comb) in the set of notches at the middle of the bed. Make sure the comb sits firmly and evenly across the bed.



At high altitudes, use

a microwave oven

to reach boiling

temperatures.

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 flask or beaker to prepare the gel solution.
- 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.
 - 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.



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60°C

DO NOT

BOILING

GEL BED.

the bed.

Hot agarose solution

may irreversibly warp

POUR

HOT AGAROSE INTO THE

Agarose Gel Preparation Step by Step Guidelines, continued

Appendix **C**

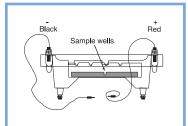
7. Cool the agarose solution to 60°C with careful swirling to promote even dissipation of heat. If detectable evaporation has occurred, add distilled water to bring the solution up to the original volume marked in step 5.

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 60° C 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.





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

c 5.	ny item is not he space must		51-5990	(301) 251-5990				% (Optional)	nication				No data	No data	No data				UEL _{N.D.}	am			
eet Communicatio oe consulted fo	permitted. If a		301) 2	(301) 2:		al)		Other Limits Recommended	zard Commu									ta	LEL N.D	standard fo			
Material Safety Data Sheet May be used to comply with OSHA's Hazard Communication Standard. 29 CRF 1910.1300 Standard must be consulted for specific requirements.	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.		Emergency Telephone Number (301) 251-5990	Telephone Number for information (3	Date Prepared 10/05/06	Signature of Preparer (optional)	Information	ACGIHTLY Reco	efined by the OSHA Ha			S	Specific Gravity (H 0 = 1)	Melting Point	Evaporation Rate (Butyl Acetate = 1)		or	ics N.D. = No data	Flammable Limits	Water spray, dry chemical, carbon dioxide, halon or standard foam	ocedures Possible fire hazard when exposed to heat or flame		
Matel sy be used to cor indard. 29 CFR 1		_	Em	_	Dat	Sign	its/Identify	OSHA PEL	materials as de			haracteristi	is	No data N	No data (E		White powder, no odor	haracteristi	Ħ	chemical, car	ard when exp		None
EDVOTEK. Sta	IDENTITY (As Used on Label and List) Agarose	Section I	Manufacturer's Name	EDVOIEK, Inc. Address (Number, Street, City, State, Zip Code)	14676 Rothgeb Drive	ROCKVIIIE, IVID 20830	Section II - Hazardous Ingredients/Identify Information	Hazardous Components [Specific Chemical Identity; Common Name(s)]	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	Vapor Pressure (mm Hg.)	Vapor Density (AIR = 1)	Solubility in Water Insoluble - cold	Appearance and Odor White p	Section IV - Physical/Chemical Characteristics	Flash Point (Method Used) No data	Extinguishing Media Water spray, dry	Special Fire Fighting Procedures Possible fire haz	Unusual Fire and Explosion Hazards	

מבנים ו	man	l				Coction V Dooctivity Data	200			
Stability	Unstable		Conditions to Avoid	pid		Section V - Neactivity	y Data	Conditions to Ausid		
	Stable >	×	None			stability	Onstable	None None	5	
Incompatibility	Strong oxidizing agents	ng agei	nts			Incompatibility No da	No data available			
Hazardous Decomposition or Byproducts Carbon monoxide, Carbon dioxide	r Byproducts Carbo	on mon	noxide, Carbon d	lioxide		Hazardous Decomposition or Byproducts	r Byproducts			
210000000000000000000000000000000000000	May Occur	Γ	Conditions to Avoid	bio						
Polymerization	Will Not Occur	×	None			Hazardous	May Occur	Conditions to Avoid		
Section VI - Health Hazard Data	Hazard Data	1				rolymenization	Will Not Occur X		None	
Route(s) of Entry:	Inhalation?	رد ج	Skin?	X	Ingestion?	Section VI - Health Hazard Data Route(s) of Entry:	Hazard Data	Caid		l pastion 2
Health Hazards (Acute and Chronic) None	d Chronic) None	1				Health Hazards (Acute and Chronic)	Yes		Yes	Yes
Carcinogenicity: None identified NTP?	antified NTP?		IARC Monographs?		OSHA Regulation?	Inhalation:	Inhalation: No data available Ingestion: Large amounts may cause diarrhea	idestion: Large amoun	mounts may c	ause diarrhea
						Calculage mary.		BOILDIN DUC		OSHA Regulation?
Signs and Symptoms of Exposure Irritation to upper respiratory tract, skin, eyes	sposure Irritation	n to up	oper respiratory	y tract, skin, €	syes	Signs and Symptoms of Exposure No data available	xposure No data avail.	lable		
Medical Conditions Generally Aggravated by Exposure	rally Aggravated by	y Expos	ure None			Medical Conditions Generally Aggravated by Exposure No data available	rally Aggravated by Expo	osure No data avail	lable	
Emergency First Aid Procedures Ingestion: If conscious, give large amounts of water	edures Ingestion	ii If	nscious, give la	arge amounts	s of water	Emargancy Eiret Aid Drocoduras	politos			
Eyes: Flush with water Inhalation: Move to fresh air	r Inhalation: Mo	ove to 1	fresh air Skin	: Wash with	Skin: Wash with soap and water			Treat symptomatically and supportively	oortively	
Section VII - Precautions for Safe Handling and Use	ions for Safe Ha	andlir	ng and Use			call bac sailbach of 2 and sacitive seal - IIV acities	Ibach Ofc Cafe	oal bac sai		
Steps to be Taken in case Material is Released for Spilled Wear stiftable protective clothing. Mop up spill and rines with water or collect in abcorraine material and discose of the abcorraine material	Material is Released	d for St We	oilled ar suitable prof material and d	tective clothii	ng. Mop up spill	Steps to be Taken in case Material is Released for Spilled Sweep up and place in sur	Material is Released for Spilled Sweep up and place in suitable container for disposal	Spilled	ainer for disp	losa
'in the same of th	0.000			0.000						
Waste Disposal Method	Dispose in accordance with all applicable federal, state, and local enviromental regulations.	dance gulatic	with all applica ons.	able federal, :	state, and local	Waste Disposal Method	Normal solid waste disposal	disposal		
Precautions to be Taken in Handling and Storing	η Handling and Stor	ring				Precautions to be Taken in Handling and Storing	n Handling and Storing			
	Avoid eye and skin contact.	kin cor	ntact.				None			
Other Precautions	None					Other Precautions	None			
Section VIII - Control Measures	Measures					Section VIII - Control Measures	Measures			
Respiratory Protection (Specify Type)	pecify Type)					Respiratory Protection (S	Respiratory Protection (Specify Type) Chemical cartridge respirator with full facepiece.	cartridge respirat	tor with full fa	acepiece.
Ventilation	Local Exhaust	Yes		Special	None	Ventilation	Local Exhaust		Special	
	Mechanical (General)	l	Yes	Other	None		Mechanical Gen. dilution ventilation	ution ventilation	Other	
Protective Gloves	Yes		Eye Protection	ection	_Safety goggles	Protective Gloves Yes		Eye Protec	ction Splash	Eye Protection Splash proof goggles
Other Protective Clothing or Equipment None	or Equipment No	one				Other Protective Clothing or Equipment Impervious clothing to prevent skin contact	Jor Equipment Impervic	ous clothing to pr	revent skin co	ntact

n or	EDVOTEK. Su	Mar lay be used to tandard. 29 CF	Material Safety Data Sheet May be used to comply with OSHA's Hazard Communication Standard. 29 CR 1910.1300 Standard must be consulted for specific requirements.	neet Communicatio be consulted fo	n vr
ny item is not ne space must	IDENTITY (As Used on Label and List) 50x Electrophoresis Buffer	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.	t permitted. If an ion is available, th	y item is not ne space must
	Section I	_			
1-5990	Manufacturer's Name		Emergency Telephone Number		(301) 251-5990
1-5990	Address (Number, Street, City, State, Zip Code)	<u> </u>	Telephone Number for information (3	mation (301) 25	(301) 251-5990
	14676 Rothgeb Drive		Date Prepared	10/05/06	
	KOCKVIIIE, INID 20850	F	Signature of Preparer (optional)	nal)	
	Section II - Hazardous Ingredients/Identify Information	nts/Identif	y Information		
% (Optional)	Hazardous Components [Specific Chemical Identity, Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
nunication	This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard	rdous mater	ials as defined by the O	SHA Hazard	
	Section III - Physical/Chemical Characteristics	Characteris	itics		
No data	Boiling Point	No data	Specific Gravity $(\frac{1}{2}0 = 1)$		No data
No data	Vapor Pressure (mm Hg.)	No data	Melting Point		No data
No data	Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)		No data
	Solubility in Water Appreciable, (g	(greater than 10%)	10%)		
	Appearance and Odor Clear, liquid, slight vinegar odor	light vinega	odor .		
	Section IV - Physical/Chemical Characteristics	Characteri	N.D. =	No data	
UEL data	Flash Point (Method Used) No data	ta	Flammable Limits	LEL N.D.	UEL N.D.
	Extinguishing Media Use	e extinguish	Use extinguishing media appropriate for surrounding fire.	for surroundi	ng fire.
ind, avoid SCBA.	Special Fire Fighting Procedures We ope	ear protectiv erated in po	Wear protective equipment and SCBA with full facepiece operated in positive pressure mode.	with full face	piece
	Unusual Fire and Explosion Hazards	None identified			

Section V - Reactivity Data	y Data			Section V - Reactivity Data	y Data	
Stability	Unstable	Conditions to Avoid	oid	Stability	Unstable	Conditions to
Incompatibility	None X	None	ne	Incompatibility	Strong oxidizing agents	ng agents
Hazardous Decomposition or Byproducts Sulfur oxides, and bromides	r Byproducts Sulfur oxid	es, and bromide	sa	Hazard ous Decomposition or Byproducts Carbon monoxide, Carbo	r Byproducts Carbo	on monoxide
Hazardous Polymerization	May Occur Will Not Occur	Conditions to Avoid None	. Avoid None	Hazardous Polymerization	May Occur	Conditions to
Section VI - Health Hazard Data	Hazard Data			Section VI - Health Hazard Data	Hazard Data	-
Route(s) of Entry:	tion?	Yes Skin?	Yes Ingestion? Yes		Inhalation?	, Yes
Health Hazards (Acute and Chronic)	onic)	Acute eye contact: May cause irritation. No data available for other routes.	ause irritation. er routes.	Health Hazards (Acute and Chronic) None	d Chronic) None	
Carcinogenicity: No data available	Ξ	IARC Monographs?	graphs? OSHA Regulation?	Carcinogenicity: None identified	entified NTP?	IARCMon
Signs and Symptoms of Exposure		May cause skin or eye irritation	tion	Signs and Symptoms of Exposure	xposure Irritation	Irritation to upper respirat
Medical Conditions Generally Aggravated by Exposure None reported	rally Aggravated by Expo	sure None repo	orted	Medical Conditions Generally Aggravated by Exposure None	rally Aggravated by	/ Exposure
Emergency First Aid Procedures		Treat symptomatically and supp with copious amounts of water.	Treat symptomatically and supportively. Rinse contacted are: with copious amounts of water.	rea Emergency First Aid Procedures Ingestion: If conscious, giv Eyes: Flush with water Inhalation: Move to fresh air S	edures Ingestior r Inhalation: Mo	i: If conscio
Section VII - Precautions for Safe Handling and Use	ions for Safe Handli	ing and Use		Section VII - Precautions for Safe Handling and Use	ions for Safe H	andling ar
Steps to be Taken in case Material is Released for Spilled Wear eye and skin protection and mop spill area	s to be Taken in case Material is Released for Spilled Wear eye and skin protection and mop spill area. Rinse with water.	ipilled Il area. Rinse w	vith water.	Steps to be Taken in case Material is Released for Spilled Wear sultable pand rinse with water or collect in absorptive material an	Material is Released or collect in abso	for Spilled Wear sui
Waste Disposal Method Observe all federal,	te Disposal Method Observe all federal, state, and local regulations.	tions.		Waste Disposal Method	Dispose in accordance with all app enviromental regulations.	dance with
Precautions to be Taken in Handling and Storing Avoid eye and skin contact.	n Handling and Storing contact.			Precautions to be Taken in Handling and Storing Avoid eye and skin o	n Handling and Storing Avoid eye and skin contact.	ring in contact.
Other Precautions None				Other Precautions	None	
Section VIII - Control Measures	Measures			Section VIII - Control Measures	Measures	
Respiratory Protection (Specify Type)	pecify Type)			Respiratory Protection (Specify Type)	pecify Type)	
Ventilation	Local Exhaust	Yes		Ventilation	Local Exhaust	Yes
	Mechanical (General)	Yes	Other None		Mechanical (General)	eral) Yes
Protective Gloves Yes	s	Eye Protection	tection Splash proof goggles	S Protective Gloves	Yes	
Other Protective Clothing or Equipment		None required		Other Protective Clothing or Equipment None	or Equipment No	ne
Work/Hygienic Practices	piony	Avoid eve and skin contact	treta			

nergency Telephone Number (301) 251-5990

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

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

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.

EDVOTEK.

phone Number for information (301) 251-5990

Address (Number, Street, City, State, Zip Code)

EDVOTEK, Inc.

14676 Rothgeb Drive Rockville, MD 20850

10/05/06

rzardous Components [Specific Other Limits of Herical Identity; Common Name(s)] OSHA PEL ACGIH TLV Recommended % (Option

Section II - Hazardous Ingredients/Identify Information

s product contains no hazardous materials as defined by the OSHA Hazard Con

Specific Gravity $(H_2^10 = 1)$

No data No data

Melting Point

Vapor Pressure (mm Hg.) por Density (AIR = 1)

3oiling Point

No data

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

Unknown

Jnusual Fire and Explosion Hazards

xtinguishing Media Dry chemical, carbon dioxide, water spray or foam

ion IV - Physical/Chemical Cr Point (Method Used) No data

Blue liquid, no odor

olubility in Water Soluble

LEL No data No data