Edvo-Kit # 101

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Principles and Practice of Agarose Gel Electrophoresis

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.

See page 3 for storage instructions.

Version 101.210628

EDVOTEK®

Table of Contents

Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets

Experiment Components

READY-TO-LOAD™ SAMPLES FOR ELECTROPHORESIS

Components (in QuickStrip™ format)		Check (✓)
Store QuickStrip™ samples in the refrigerator upon receipt.		
A	Orange	❑
B	Purple	❑
C	Red	❑
D	Blue 1	❑
E	Dye Mixture	❑
F	Blue Dye Mixture (Blue 1 + Blue 2)	❑

Experiment #101 is designed for 8 groups.

Store QuickStrip™ samples in the refrigerator immediately upon receipt. All other components can be stored at room temperature.

REAGENTS & SUPPLIES

Store the following at room temperature.

- Ultra Spec-Agarose™ ❑
- Electrophoresis Buffer (50x) ❑
- Practice Gel Loading Solution ❑

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipettes with tips
- Balance
- Microwave, hot plate or burner
- Pipet pump
- 250 mL flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- DNA visualization system (white light)
- Distilled or deionized water

All experiment 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.

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, Ultra-Spec 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.

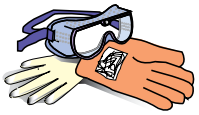
Experiment Overview

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.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.



Wear gloves and safety goggles

LABORATORY NOTEBOOKS

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

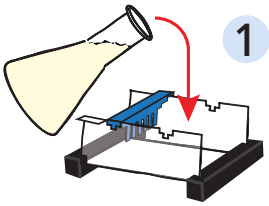
After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

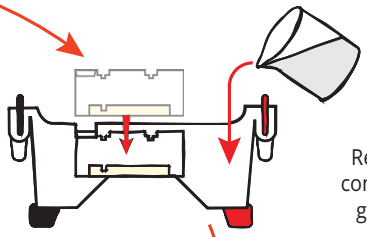
Experiment Overview

Agarose Gel Electrophoresis

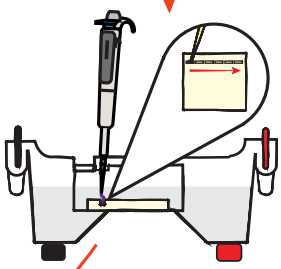
Time required: See Table C



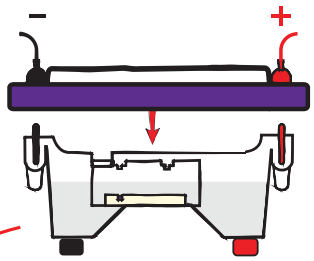
1 Prepare agarose gel in casting tray.



2 Remove end caps & comb, then submerge gel under buffer in electrophoresis chamber.

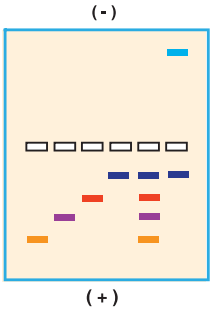


3 Load each sample in consecutive wells



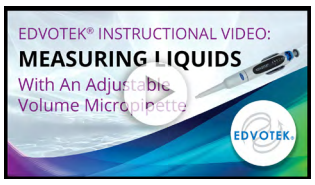
4 Attach safety cover & connect leads to power source to conduct electrophoresis.

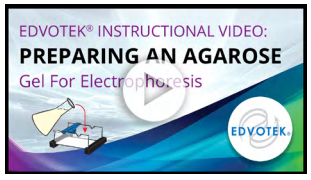
Quick Reference for EDVO-Kit #101			
Size of gel casting tray	*Groups per gel*	*Placement of comb*	*Wells required per group*
10 x 7 cm	1 group	Middle set of notches	6
14 x 7 cm	1 group	Middle set of notches	6

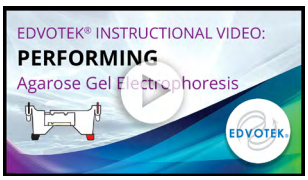


5 Analysis on white light source.

Related EDVOTEK® Instructional Videos

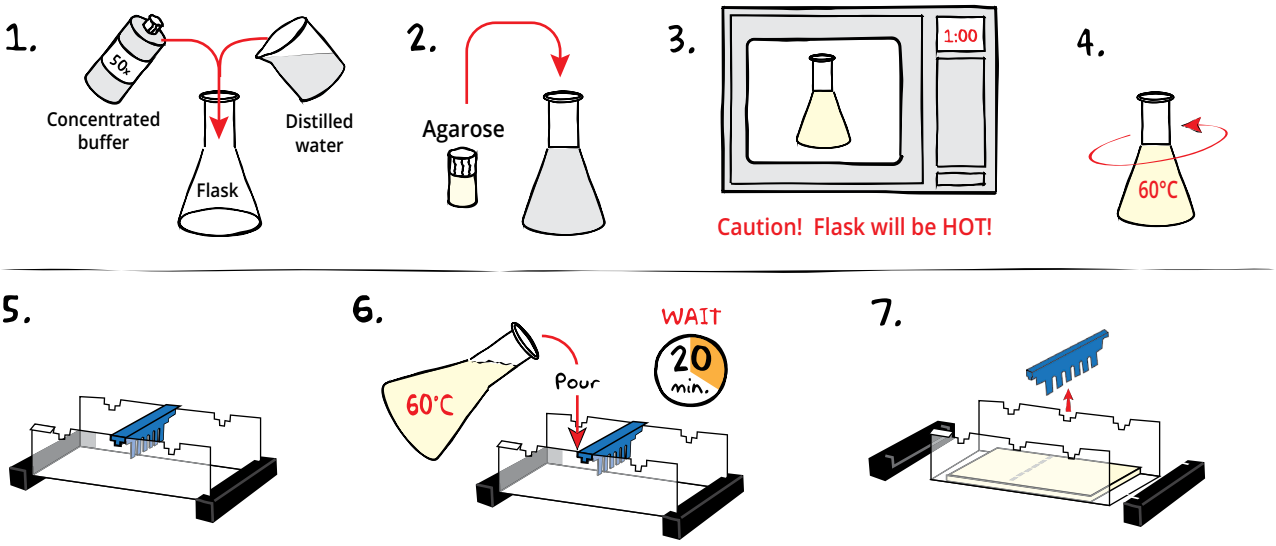






www.youtube.com/edvotekinc

Agarose Gel Electrophoresis



CASTING THE AGAROSE GEL

1. **DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
2. **MIX** agarose powder with buffer solution in a 250 mL flask (refer to Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60 °C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Wear gloves and safety goggles

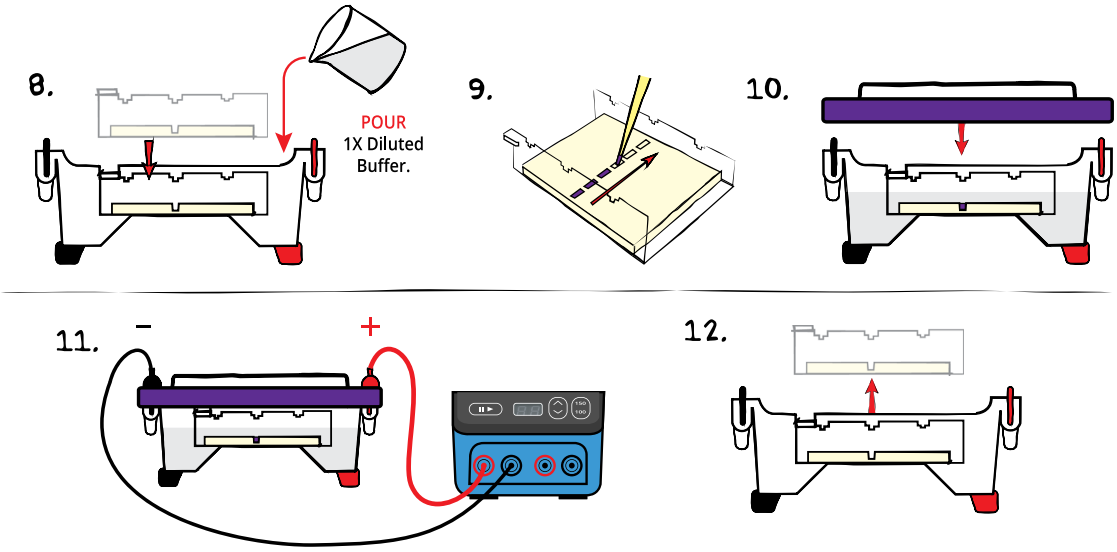
REMINDER:
This experiment requires 0.8% agarose gels cast with 6 wells.

Table A — **Individual 0.8% UltraSpec-Agarose™ Gels**

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
7 x 7 cm	0.6 mL	29.4 mL	0.24 g	30 mL
10 x 7 cm*	0.9 mL	44.1 mL	0.36 g	45 mL
14 x 7 cm	1.2 mL	58.8 mL	0.48 g	60 mL

**Recommended gel volume for the EDGE™ Integrated Electrophoresis System. (Cat. #500).*

Agarose Gel Electrophoresis, continued



RUNNING THE GEL

8. **PLACE** the gel (still on the tray*) into the electrophoresis chamber. **COVER** the gel with 1X Electrophoresis Buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **PUNCTURE** the foil overlay of the QuickStrip™ with a pipet tip. **LOAD** the entire sample (35 µL) into the well in the order indicated by Table 1, at right.
10. **PLACE** safety cover on the unit. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines). Allow the tracking dye to migrate at least 3 cm from the wells.
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and proceed to **VISUALIZING** the agarose gel.

NOTE: Dyes do not require staining. Analyze and document results immediately following gel electrophoresis. Dyes will diffuse and eventually fade from the gel.

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

TABLE 1: GEL LOADING

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

Table B — **1x Electrophoresis Buffer (Chamber Buffer)**

EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
EDGE™	150 mL	3 mL	147 mL
M12	400 mL	8 mL	392 mL
M36	1000 mL	20 mL	980 mL

Table C — **Time and Voltage Guidelines (0.8% Agarose Gel)**

	Electrophoresis Model: EDGE™	M12 & M36
Volts	Min/Max (minutes)	Min/Max (minutes)
150	10/20	20/35
125	N/A	30/45
100	15/25	40/60

*Gels that have previously been removed from their trays should be "anchored" back to the tray with a few drops of molten agarose before placing into the electrophoresis chamber. This will prevent the gels from sliding around in the trays and the chambers.

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?

Instructor's Guide

ADVANCE PREPARATION:

PREPARATION FOR:	WHAT TO DO:	WHEN?	TIME REQUIRED:
Agarose Gel Electrophoresis	Prepare QuickStrips™.	Up to one day before performing the experiment.	45 min.
	Prepare diluted electrophoresis buffer.		
	Prepare molten agarose and pour gels.		

NOTE:
Accurate pipetting is critical for maximizing successful experiment results. EDVOTEK Series 100 experiments are designed for students who have had previous experience with micropipetting techniques and agarose gel electrophoresis.

If students are unfamiliar with using micropipettes, we recommended performing **Cat. #S-44, Micropipetting Basics** or **Cat. #S-43, DNA DuraGel™** prior to conducting this experiment.

Pre-Lab Preparations: Module I

AGAROSE GEL ELECTROPHORESIS

This experiment requires 0.8% agarose gels. Enough reagents are provided to cast either eight 10 x 7 cm gels or four 14 x 7 cm gels with the well template (comb) in the middle set of notches. You can choose whether to prepare the gels in advance or have students prepare their own. Allow approximately 30 minutes for this procedure.

Each group will need:

- 50x concentrated buffer
- Distilled Water
- Ultra Spec-Agarose™
- QuickStrip™ Samples

Quick Reference for EDVO-Kit #101

Size of gel casting tray	*Groups per gel*	*Placement of comb*	*Wells required per group*
10 x 7 cm	1 group	Middle set of notches	6
14 x 7 cm	1 group	Middle set of notches	6

Individual Gel Preparation:
Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See Student's Experimental Procedures. Students will need 50x concentrated buffer, distilled water and agarose powder.

Batch Gel Preparation:
To save time, a larger quantity of agarose solution can be prepared for sharing by the class. Electrophoresis buffer can also be prepared in bulk. See Appendix B.

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 freeze gels at -20 °C as freezing will destroy the gels.

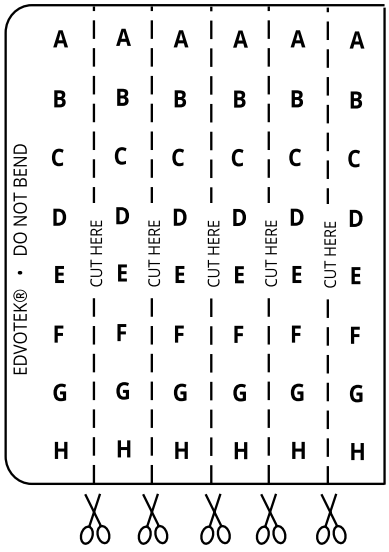
Gels that have been removed from their trays for storage should be "anchored" back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

SAMPLES FORMAT: PREPARING THE QUICKSTRIPS™

QuickStrip™ tubes consist of a microtiter block covered with a protective foil overlay. Each well contains pre-aliquoted sample.

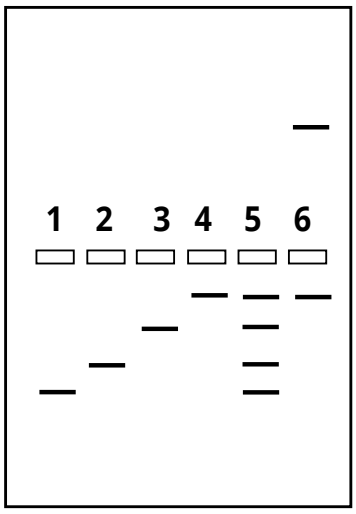
Using sharp scissors, carefully divide the block of tubes into individual strips by cutting between the rows (see diagram at right). Take care not to damage the foil overlay while separating the samples.

Each lab group will receive one set of tubes. Before loading the gel, remind students to tap the tubes to collect the sample at the bottom of the tube. Puncture the foil overlay of the QuickStrip™ with a pipet tip to aspirate the sample. ***Do not remove the foil as samples can spill.***



Carefully cut between each set of tubes

Experiment Results and Analysis



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

Lanes 1, 2, 3, and 4 each contain a single dye, which will form a unique band on the gel. Lane 5 contains a mixture of all 4 dyes. Lane 6 contains two blue dyes that migrate in opposite directions on the gel - Blue 1 is negatively charged while Blue 2 is positively charged and will move towards the negative electrode.

Results Table	
Lane 1	Orange
2	Purple
3	Red
4	Blue 1
5	Dye Mixture
6	Blue Dye Mixture (Blue 1 + Blue 2)

Questions and Answers to Study Questions

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?

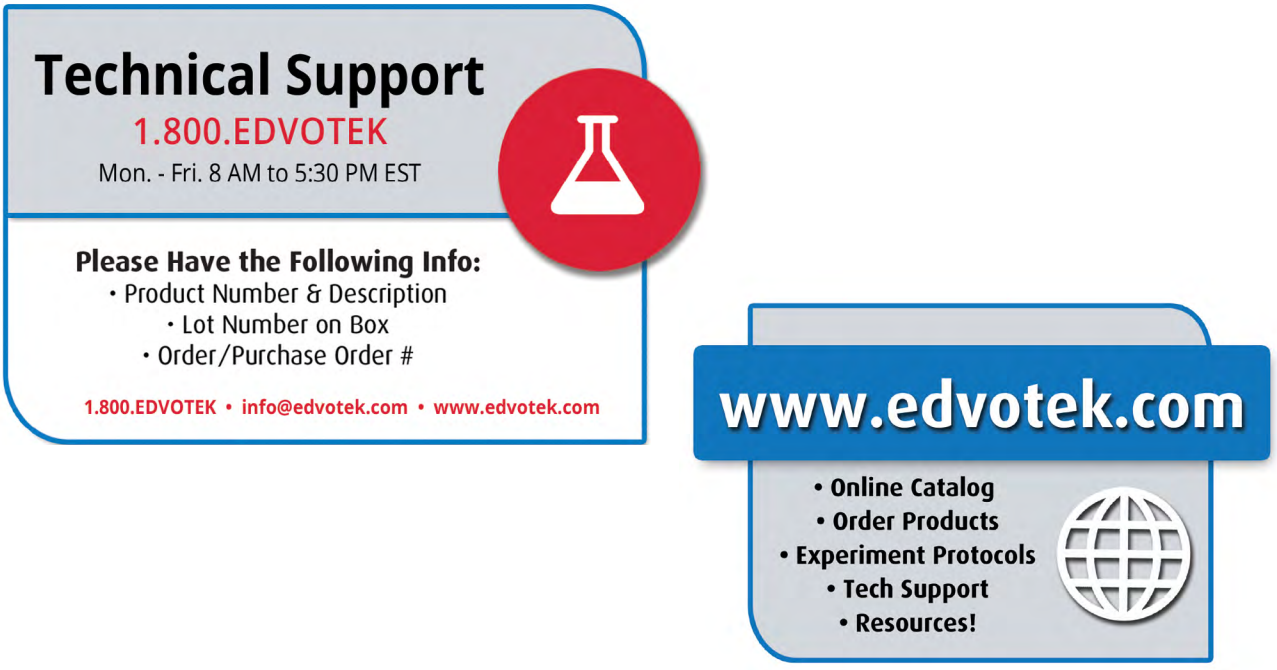
No ions are contained in distilled water. Ions are required for conductivity of the fluid and therefore, the ability of the molecules to migrate through the gel.

Appendices

A EDVOTEK® Troubleshooting Guide

B Bulk Preparation of Electrophoresis Buffer and Agarose Gels

Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets



Appendix A
EDVOTEK® Troubleshooting Guides

PROBLEM:	CAUSE:	ANSWER:
Bands are not visible on the gel.	The gel was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
Bands were not resolved.	Dyes should migrate at least 3.5 cm (if using a 10x7 cm tray), and at least 6 cm (if using a 7x14 cm tray) from the wells to ensure adequate separation.	Be sure to run the gel at least 6 cm (approximately one hour at 125 V).
There is no separation between bands.	The wrong percent gel was used for electrophoretic separation.	Be sure to prepare the correct percent agarose gel. For reference, the Ready-to-Load™ Dye samples should be analyzed using a 0.8% agarose gel.
I can't see the positively charged dye on the gel.	The dye ran off of the gel.	The comb was placed in the wrong position. Be sure to place the comb in the middle notches in the gel casting tray.
There's not enough sample in my QuickStrip™.	The QuickStrip™ has dried out.	Add 40 µL water, gently pipet up and down to mix before loading.

Visit www.edvotek.com for additional troubleshooting suggestions.

Appendix B

Bulk Preparation of Electrophoresis Buffer and Agarose Gels

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.

Bulk Electrophoresis Buffer

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

Table D

Bulk Preparation of Electrophoresis Buffer			
50x Conc. Buffer	+	Distilled Water	Total Volume Required
60 mL		2,940 mL	3000 mL (3 L)

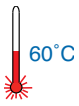
Batch Agarose Gels (0.8%)

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

Table E

Batch Prep of 0.8% UltraSpec-Agarose™				
Amt of Agarose	+	Concentrated Buffer (50X)	+ Distilled Water	Total Volume
3.0 g		7.5 mL	367.5 mL	375 mL

1. Use a 500 mL flask to prepare the diluted gel buffer.
2. Pour 3.0 grams of Ultra Spec-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. Measure 45 mL for a 10 x 7 cm tray or 60 mL for a 14 x 7 cm tray. ***For this experiment, 10 x 7 cm or 14 x 7 cm gels are recommended.***
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Solidified gels can be stored under buffer in the refrigerator for up to 2 weeks. Do not freeze gels.

NOTE:
The Ultra Spec-Agarose™ kit component is usually labeled with the amount it contains. 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.

PROCEED to Loading and Running the Gel (page 8).