

EDVOTEK® • The Biotechnology Education Company®

Edvo-Kit #

111

Edvo-Kit #111

Electrophoretic Properties of Native Proteins

Experiment Objective:

The objective of this experiment is to develop a general understanding of the structure and electrophoretic migration of native proteins.

See page 3 for storage instructions.

Version 111.190917

EDVOTEK®

1.800.EDVOTEK • www.edvotek.com • info@edvotek.com

Table of Contents

	Page
Experiment Components	3
Experiment Requirements	3
Background Information	4
Experiment Procedures	
Experiment Overview	6
Module I: Performing Electrophoresis	8
Module II: Staining Agarose Gels	10
Study Questions	11
Instructor's Guidelines	12
Pre-Lab Preparations	13
Experiment Results and Analysis	15
Answers to Study Questions	16
Appendices	17
A Practice Gel Loading	18
B Bulk Electrophoresis Preparation	19

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

Technical Support

1.800.EDVOTEK
Mon. - Fri. 8 AM to 5 PM ET



Please Have the Following Info:

- Product Number & Description
- Lot Number on Box
- Order/Purchase Order #

1.800.EDVOTEK • info@edvotek.com • www.edvotek.com

www.edvotek.com

- Online Catalog
- Order Products
- Experiment Protocols
- Tech Support
- Resources!



EDVOTEK and The Biotechnology Education Company are registered trademarks of EDVOTEK, Inc. FlashBlue, DuraGel, EDGE, and UltraSpec-Agarose are trademarks of EDVOTEK, Inc.



1.800.EDVOTEK • Fax 202.370.1501 • info@edvotek.com • www.edvotek.com

Duplication of any part of this document is permitted for non-profit educational purposes only. Copyright © 1989-2022 EDVOTEK, Inc., all rights reserved. Version 111.190917

Experiment Components

Components	Storage	Check (✓)
A Bovine Serum Albumin (BSA)	-20°C	<input type="checkbox"/>
B Ovalbumin	-20°C	<input type="checkbox"/>
C Cytochrome C	-20°C	<input type="checkbox"/>
D Lysozyme	-20°C	<input type="checkbox"/>
E Horse Serum Proteins	-20°C	<input type="checkbox"/>
• Practice Gel Loading Solution	Room Temp.	<input type="checkbox"/>
• UltraSpec-Agarose™ Powder	Room Temp.	<input type="checkbox"/>
• Electrophoresis Buffer (50x)	Room Temp.	<input type="checkbox"/>
• FlashBlue™ Protein Stain Powder	Room Temp.	<input type="checkbox"/>
Supplies		
• Transfer Pipets		<input type="checkbox"/>
• 1 mL Pipet		<input type="checkbox"/>

Quick Reference:

There is enough sample for 6 gels if you are using an automatic micropipette for sample delivery. Use of transfer pipets will yield fewer gels.

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipettes with tips
- Microwave or hot plate
- 250 mL flasks
- Safety goggles and disposable laboratory gloves
- Hot gloves
- Marking pens
- Distilled or deionized water

For Staining with FlashBlue™ Protein Stain (optional):

- White Vinegar
- Ethanol (95% or higher)
- 750 mL or 1 L flask or beaker
- Small plastic tray or large weigh boat
- Plastic wrap
- White light box (recommended)
- Rocking platform (recommended)

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

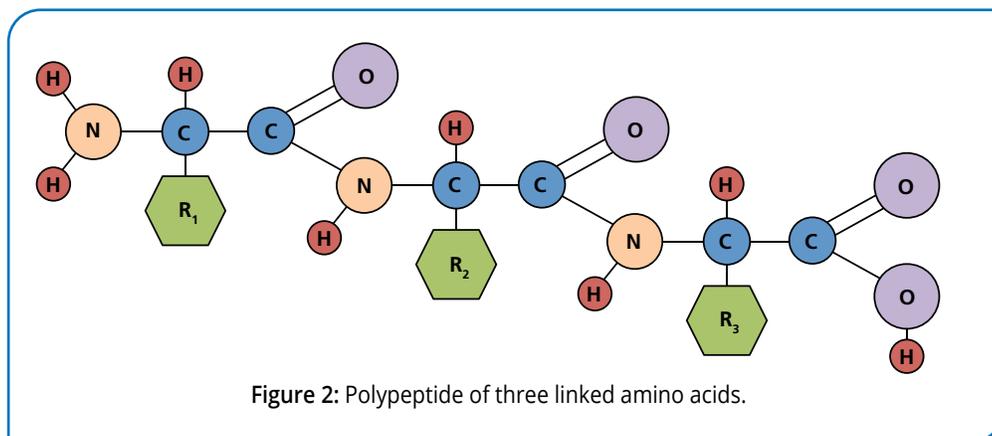
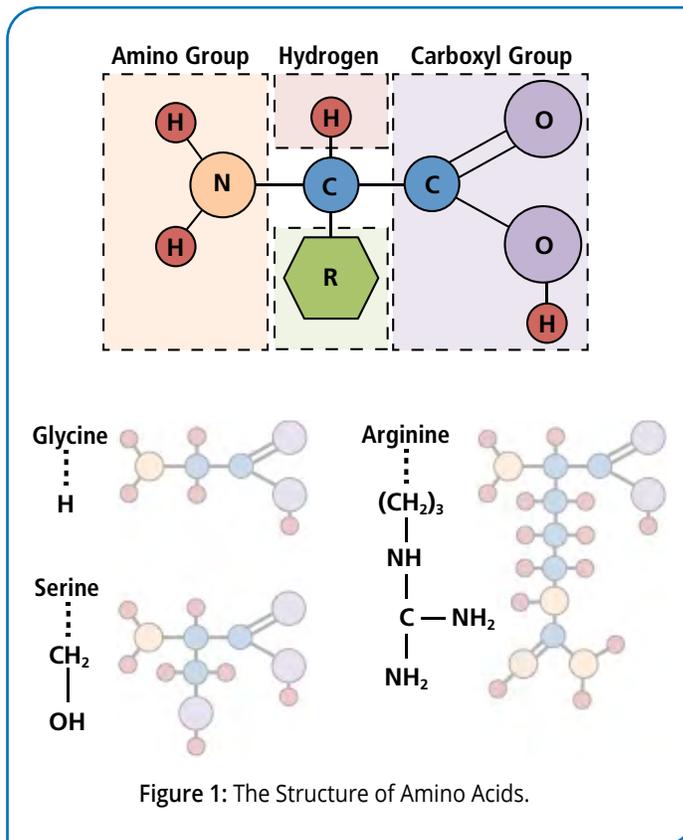
Proteins are a diverse group of large and complex molecules. Scientists first observed these molecules in the 18th century and quickly determined that they helped maintain the body's structure. Since then, it has become clear that proteins also play a role in most cellular processes. For example, proteins known as enzymes enable many of the chemical reactions essential to life. Proteins also facilitate:

- Communication between cells, organs, and tissue
- The regulation of internal pH and fluid levels
- The storage and transport of atoms and small molecules
- The growth and maintenance of most tissues
- The defense of the body against bacteria and viral intruders

Given this utility, it is not surprising that our bodies are packed with proteins - around 3 million proteins per cubic micron of cells!

Proteins are polymers that are composed of hundreds to thousands of smaller subunits known as amino acids. Amino acids are simple molecules consisting of a central carbon atom bonded to four different groups: an amine group, a carboxyl group, a hydrogen atom, and a unique side chain (Figure 1). These side chains range from simple to complex and give each amino acid unique properties. While there are hundreds of different amino acids, most proteins are constructed from twenty common ones. However, because these twenty amino acids can be arranged in many different combinations it is still possible for cells to create thousands of proteins.

During protein synthesis, the DNA code is used to create a specific sequence of amino acids which are then connected to form a continuous chain. Adjacent amino acids in the chain are linked to each other by peptide bonds. These strongly covalent bonds link the carboxyl group of one amino acid and the amine group of a second amino acid (Figure 2). A chain of linked amino acids is known as a polypeptide. Proteins can consist of a single polypeptide or several poly-



peptides. Some proteins also contain non-amino acid chemical groups. These “prosthetic” groups are usually tightly bound to one of the protein’s polypeptide chains.

The amino acid sequence gives each protein its specific properties. For example, a protein’s molecular weight is primarily determined by the number and type of amino acids in the polypeptide chain(s). While its shape - a three-dimensional configuration which includes complex twists and folds - is determined by the type, order, and number of amino acids and by certain post-translational processes. When a protein loses this folding pattern - due to exposure to heat, acids, detergents, or certain organic solvents - it also loses its biological activity and functionality. Such proteins are called denatured. In contrast, proteins that have their 3-D shape are called native proteins.

Another important property of a protein is its charge. This charge is determined primarily by what amino acids are present, where different amino acids are located within the molecule, and the pH of the solution that the protein is in. The common structure of all amino acids - the amine group, carbon atom, and carboxyl group - form a special type of dipolar molecule that is negatively charged on one side and positively charged on the other. This type of molecule has one of the best names in chemistry - a zwitterion! It also means that the molecule can be negative, positive, or neutral depending on the pH of the surrounding environment. An amino acid’s charge is also determined by its side chain. In fact, amino acids are classified by whether their side-chain makes them basic, acidic, polar but uncharged, or unpolar and uncharged. There are three basic amino acids (lysine, arginine, and ornithine) and the two acidic amino acids (glutamic acid and aspartic acid). In general, a protein molecule with a high lysine, arginine, or ornithine content will have an overall positive charge while a protein with a high glutamic or aspartic acid content will have an overall negative charge.

Gel electrophoresis can be used to examine and characterize protein diversity. This is because key protein properties - such as charge, size, and shape - affect the way that these molecules move through a gel. During electrophoresis samples containing thousands of protein molecules are carefully loaded into an agarose or polyacrylamide gel. Next, a current is applied. Proteins with a net positive charge will migrate through the gel towards the negative anode while proteins with a net negative charge will migrate through the gel towards the positive cathode. Moreover, proteins with a strong charge will migrate faster than proteins with a weak charge. This separates proteins according to both the type and strength of their charge. The migration rate of a protein is also determined by its size and shape. At a microscopic level, both agarose and polyacrylamide gels are networks of similarly sized pores that act as a molecular sieve. Small and compact molecules more easily fit through these pores and so migrate further through a gel than larger or more expansive molecules.

Electrophoresis of native proteins is useful in the clinical and immunological analysis of complex biological samples, such as serum. For example, the electrophoretic patterns of human serum proteins can aid in the diagnosis of certain diseases such as cirrhosis of the liver, certain cancers of the immune system and chronic rheumatoid arthritis. Serum consists of many different types of proteins. By running an electrophoresis test of a patient’s serum, doctors can distinguish between these different proteins and observe if any are present in abnormally high or low amounts.

In this experiment you will run four common proteins - Bovine Serum Albumin, Ovalbumin, Cytochrome C, and Lysozyme - to determine their charge and relative size. (Because most denaturation processes cause proteins to also lose their charge, these four proteins are in their native form. In addition, you will run a complex sample of horse serum. All samples also contain glycerol, which helps when loading the samples into the gel, and a negatively charged bromophenol blue tracking dye. After electrophoresis, the proteins will be visualized by staining. In the stained gel, proteins will appear as dark blue zones against a light blue background.

Experiment Overview

EXPERIMENT OBJECTIVE

The objective of this experiment is to develop a general understanding of the structure and electrophoretic migration of native proteins.

LABORATORY SAFETY

- Wear gloves and goggles while working in the laboratory.
- Exercise caution when working in the laboratory – you will be using equipment that can be dangerous if used incorrectly.
- Wear protective gloves when working with hot reagents like boiling water and melted agarose.
- Always wash hands thoroughly with soap and water after working in the laboratory.



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:

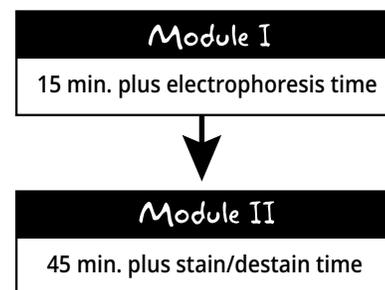
- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

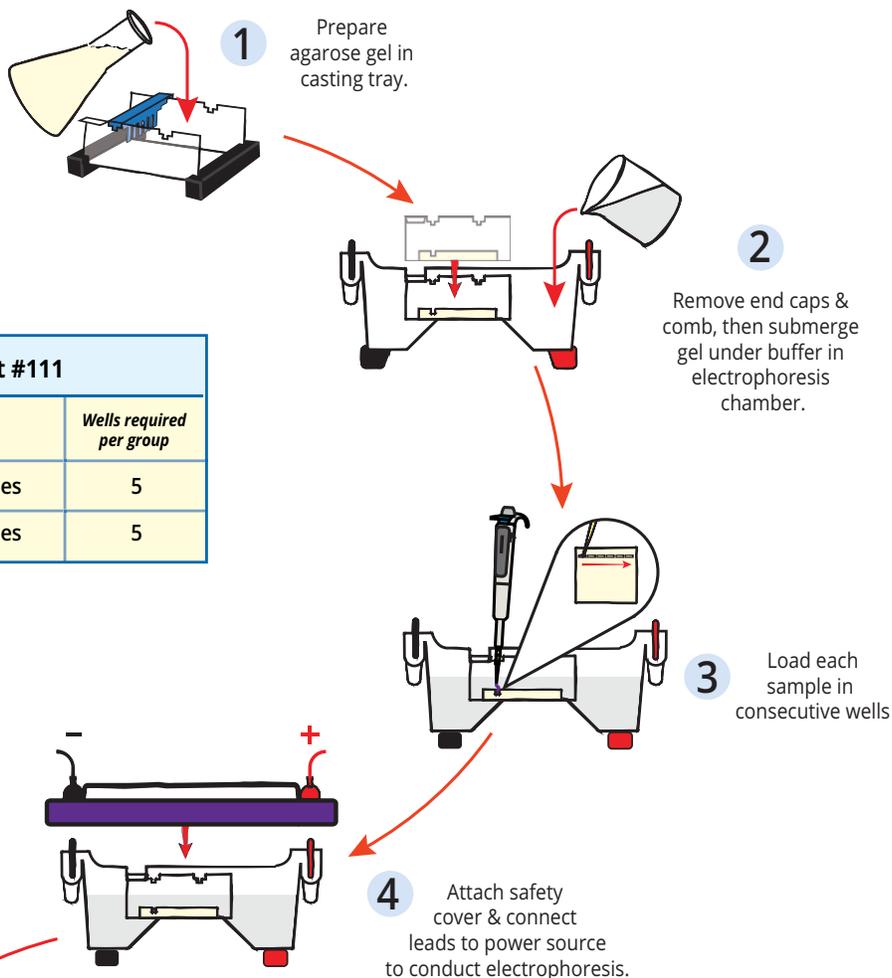
- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.



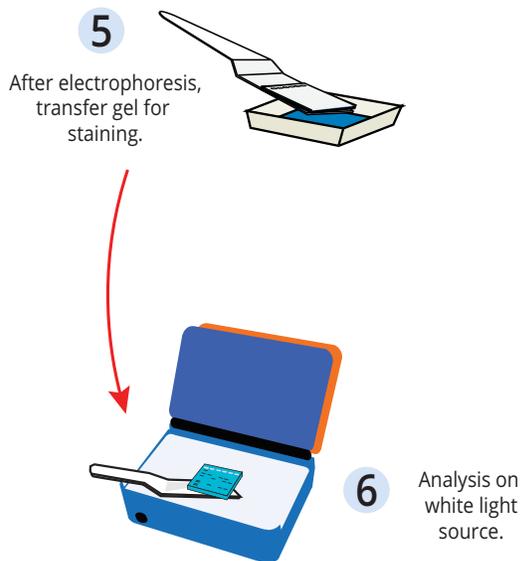
Experiment Overview

MODULE I: Agarose Gel Electrophoresis

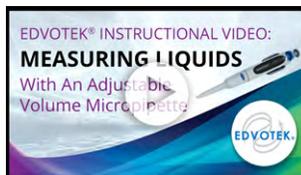
Quick Reference for EDVO-Kit #111			
Size of gel casting tray	Groups per gel	Placement of comb	Wells required per group
10 x 7 cm	1 group	Middle set of notches	5
14 x 7 cm	1 group	Middle set of notches	5



MODULE II: Staining Agarose Gels

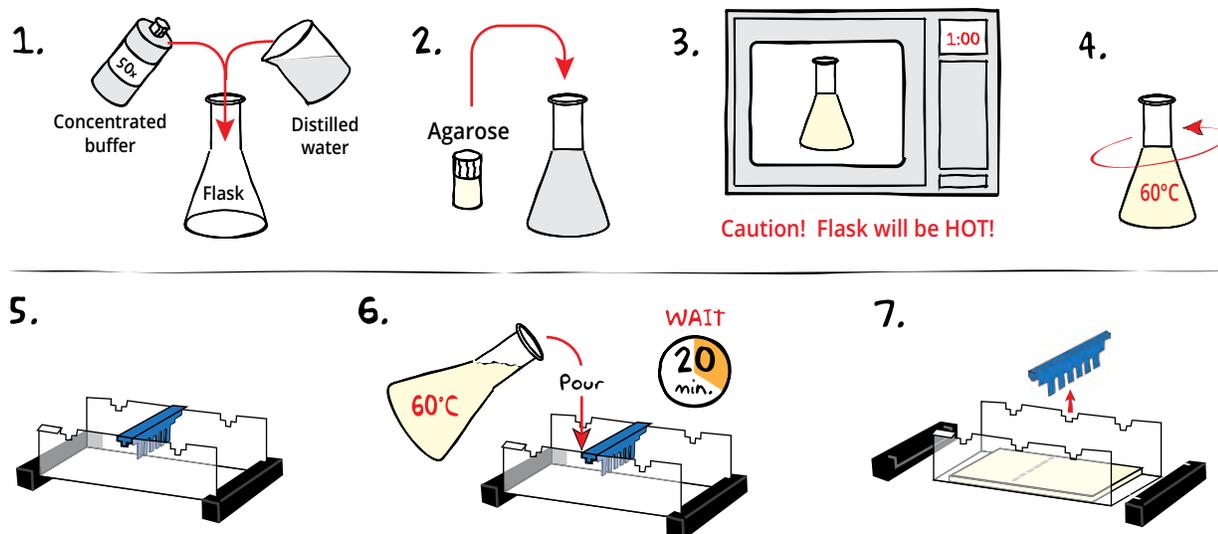


Related EDVOTEK® Instructional Videos



www.youtube.com/edvotekinc

Module I: Agarose Gel Electrophoresis



CASTING THE AGAROSE GEL

- DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
- MIX** agarose powder with buffer solution in a 250 mL flask (refer to Table A).
- 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).
- COOL** agarose to 60°C with careful swirling to promote even dissipation of heat.
- 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.
- 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.
- 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 a 7x10 cm or a 7x14 cm gel. Place well template (comb) in the middle set of notches.

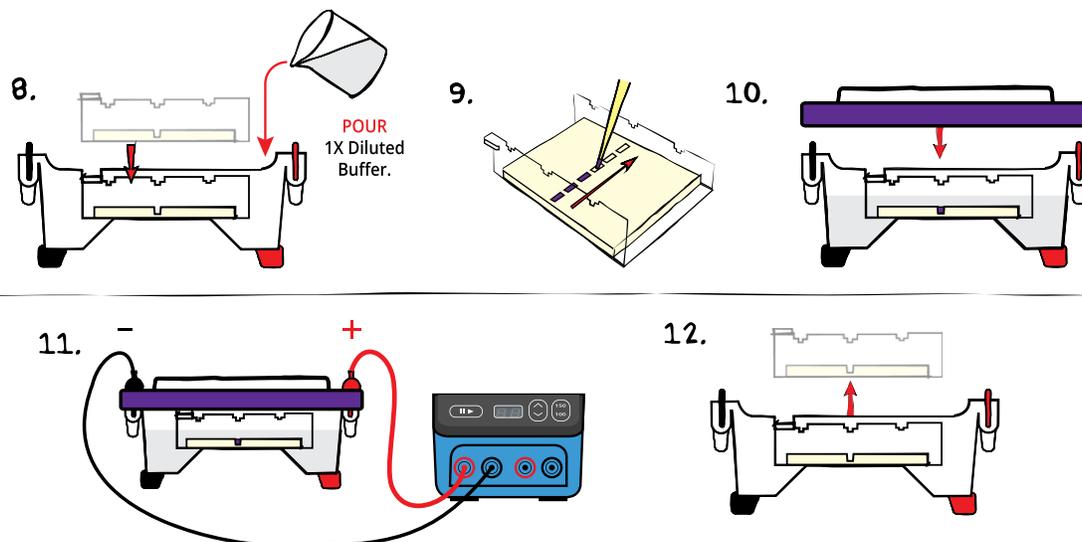
Table
A

Individual 0.8% UltraSpec-Agarose™ Gels

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
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).

Module I: 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. **LOAD** the entire sample (40 μ L) into the well in the order indicated by Table 1.
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 instructions for **STAINING** the agarose gel.

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

Table 1: Gel Loading

Lane	Tube	Sample
1	Tube A	Bovine Serum Albumin (BSA)
2	Tube B	Ovalbumin
3	Tube C	Cytochrome C
4	Tube D	Lysozyme
5	Tube E	Horse Serum Proteins

Table B
1x Electrophoresis Buffer (Chamber Buffer)

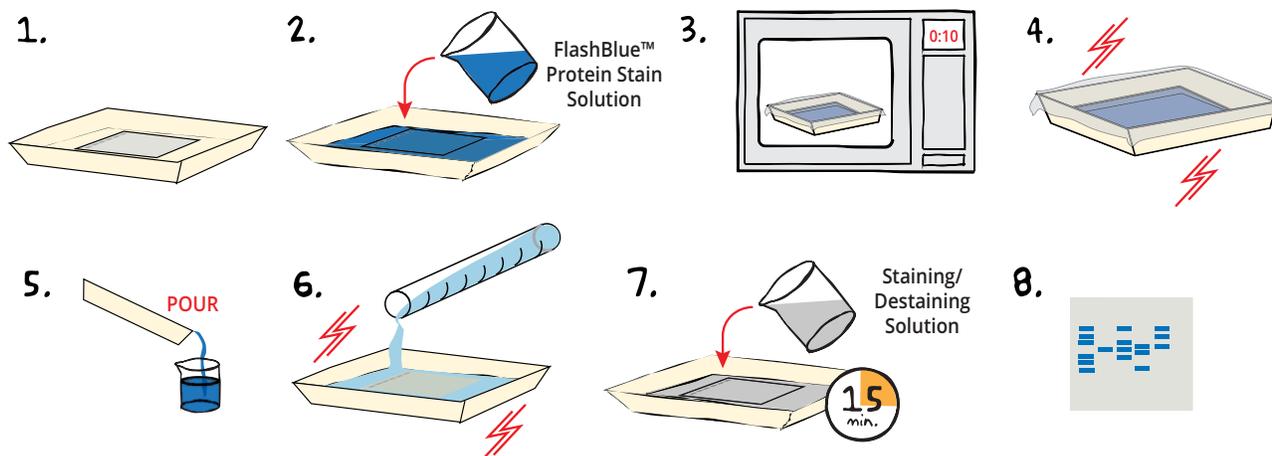
EDVOTEK Model #	Total Volume Required	Dilution	
		50x Conc. Buffer	+ Distilled Water
EDGE™	150 mL	3 mL	147 mL
M12	400 mL	8 mL	392 mL
M36	1000 mL	20 mL	980 mL

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

Volts	Electrophoresis Model	
	EDGE™	M12 & M36
	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.

Module II: Staining Agarose Gels



1. **PLACE** the gel into a shallow container.
2. **ADD** 30 mL of prepared FlashBlue™ Protein Stain.
3. (OPTIONAL) **COVER** the container with plastic wrap and **MICROWAVE** for 10 seconds to gently heat the solution.
4. **INCUBATE** for 15 minutes at room temperature, **SHAKING** occasionally.
5. **DISCARD** the FlashBlue™ Protein Stain solution. *NOTE: Pour slowly to keep the gel in the container.*
6. **WASH** the gel by partially filling container with water and gently rocking back and forth several times. **DISCARD** the used water and **REPEAT** with fresh water.
7. **ADD** 30 mL of Protein Staining/Destaining solution to the gel and **INCUBATE** for 15 minutes at room temperature.
8. **EXAMINE** the gel.
9. (OPTIONAL) **DISCARD** the used Protein Staining/Destaining solution and **ADD** an additional 30 mL of Protein Staining/Destaining solution. **INCUBATE** for 15-60 minutes at room temperature until the appearance and contrast of the protein bands against the background improves.

After staining, protein bands will appear medium to dark blue against a light background. A white light box can be used to better visualize the protein bands.

Study Questions

1. What is an amino acid? Draw or describe the four subparts of an amino acid.
2. In what direction would you predict a protein composed of mostly lysine, arginine, and ornithine amino acids to move during electrophoresis?
3. What would happen in a native protein was run in a gel next to a denatured sample of the same protein?

Instructor's Guide

ORGANIZING AND IMPLEMENTING THE EXPERIMENT

Prior to starting this experiment, carefully check the list of components and requirements on page 3 to ensure you have all the necessary components and equipment.

This experiment is supplied with enough sample for 6 gels if you are using an automatic micropipette for sample delivery. Use of transfer pipets will yield fewer gels.

Preparation for:	What to do:	When?	Time Required:
Module I: Agarose Gel Electrophoresis	Prepare diluted electrophoresis buffer	Up to one day before performing the experiment.	10-45 min.
	Prepare molten agarose and pour gels		
	Aliquot protein samples		
Module II: Staining Agarose Gels	Prepare staining components	Up to one week before the class period.	10 min.

Technical Support

1.800.EDVOTEK

Mon. - Fri. 8 AM to 5 PM ET



Please Have the Following Info:

- Product Number & Description
- Lot Number on Box
- Order/Purchase Order #

1.800.EDVOTEK • info@edvotek.com • www.edvotek.com

www.edvotek.com

- Online Catalog
- Order Products
- Experiment Protocols
- Tech Support
- Resources!



Pre-Lab Preparations

MODULE I

Preparing Agarose Gels for Electrophoresis

This experiment requires 0.8% agarose gels. Enough reagents are provided to cast 6 gels. You can choose whether to prepare the gels in advance or have students prepare their own. Allow approximately 30 minutes for this procedure.

Quick Reference for EDVO-Kit #111			
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	5
14 x 7 cm	1 group	Middle set of notches	5

Individual Gel Preparation:

Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See Module I in the Student's Experimental Procedure. 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.

FOR MODULE I Each group will need:

- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™
- Prepared samples & standard protein marker

Reconstitute Proteins and Standard Protein Marker

1. Vortex or flick tubes vigorously to mix.
2. Either place vortexed proteins in their original tubes in a central location to be shared by the class or aliquot 25 µL of each protein into six appropriately labeled new tubes. The latter will require 24 additional small tubes.

NOTE: Proteins may be kept at room temperature for immediate use or frozen until needed.

Pre-Lab Preparations

MODULE II

Prepare Staining Solutions

1. Prepare a stock solution of white vinegar and Ethanol* by combining 400 mL white vinegar with 200 mL Ethanol. Gently mix. Label as "Staining/Destaining Solution".
2. Add 180 mL of the Staining/Destaining Solution to a 250 mL flask or beaker. Add the entire contents of the FlashBlue™ Protein Stain powder and briefly stir or shake to mix. Residual powder can be rinsed from the tube using an additional 1 mL of Staining/Destaining Solution.
3. Store both solutions at room temperature until needed.

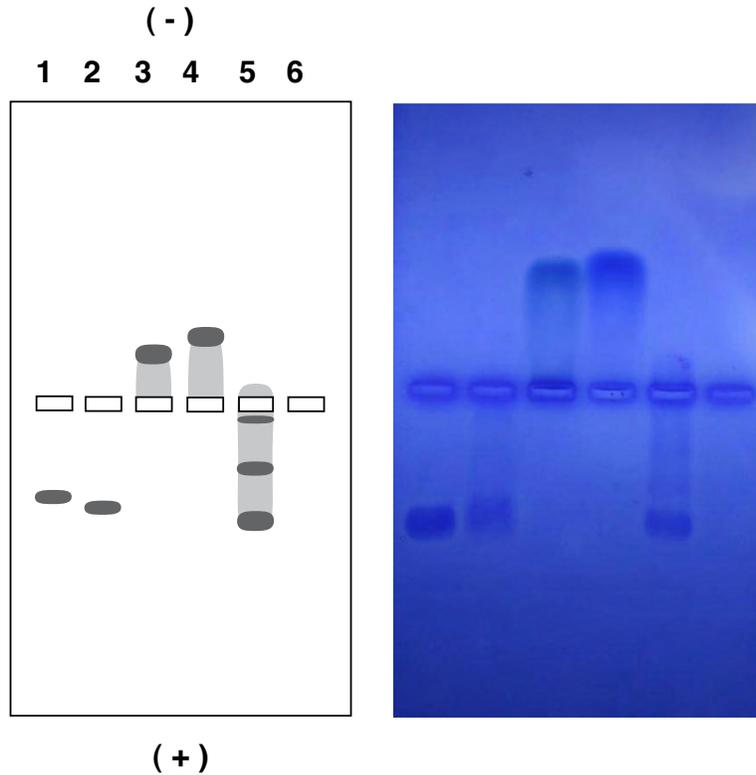
**White vinegar, sometimes called distilled or spirit vinegar, is an easy to find cooking and cleaning vinegar with an acetic acid concentration between 5-8% and a pH ~2.6. Ethanol is a common lab supply which is available at various concentrations. Our FlashBlue™ Protein Stain has been designed to work with a wide range of white vinegars. However, we do recommend using 95% Ethanol or higher.*

FOR MODULE II Each group will need:

- 30 mL FlashBlue™ Protein Stain
- 30-60 mL Staining/Destaining Solution
- Water
- Staining Tray
- Plastic Wrap
- Rocking platform (recommended)
- White light (recommended)

Experiment Results and Analysis

The figure below to the left is an idealized schematic showing relative positions of protein polypeptides. This idealized schematic shows the relative positions of the bands, but are not depicted to scale. Actual results are shown in the image at right.



Protein	Size	Shape	Isoelectric Point <i>(a measure of potential charge)</i>
Bovine Serum Albumin	68,000	Spherical	4.7
Ovalbumin	43,000	Spherical	4.6
Cytochrome C	12,000	Spherical	10.7
Lysozyme	14,000	Spherical	11.2

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

Appendices

- A Practice Gel Loading
- B Bulk Electrophoresis Preparation

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

Technical Support

1.800.EDVOTEK

Mon. - Fri. 8 AM to 5:30 PM EST



Please Have the Following Info:

- Product Number & Description
- Lot Number on Box
- Order/Purchase Order #

1.800.EDVOTEK • info@edvotek.com • www.edvotek.com

www.edvotek.com

- Online Catalog
- Order Products
- Experiment Protocols
- Tech Support
- Resources!



Appendix A

Practice Gel Loading

If your students are unfamiliar with loading samples in agarose we suggest they practice the delivery techniques before performing this experiment. Below is one suggested activity for practice gel loading. Although the same gel can be used for both this activity and the actual experiment we suggest using a separate gel in case of damage.

1. Cast a separate practice gel with the maximum number of combs in it. For practice gel loading you can use any gel grade agar at any concentration or you can use Edvotek's reusable DuraGels™ ([Cat. #S-43](#)).
2. Place the gel under water or buffer either in the chamber or in a similarly deep tray.
3. Let students practice delivering the practice gel solution to the sample wells.
4. If the students need more practice, remove the practice gel loading solution by squirting buffer into the wells with a transfer pipet.

Appendix B

Bulk Electrophoresis Preparation

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.

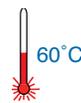
Table D Bulk Preparation of Electrophoresis Buffer			
50x Conc. Buffer	+	Distilled Water	Total Volume Required
60 mL		2,940 mL	3000 mL (3 L)

Batch Agarose Gels (0.8%)

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

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. Measure 45 mL for a 10 x 7 cm tray or 60 mL for a 14 x 7 cm tray.
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.

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



NOTE:

The UltraSpec-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 9).