Edvo-Kit # 1110

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Cell Types in the Brain

Experiment Objective:

The objective of the experiment is for students to examine the differences between cell types in the brain based on their protein profiles.

See page 3 for storage instructions.

Version 1110.220222

EDVOTEK®

Table of Contents

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

Experiment Components

Component		Storage	Check ✓
A	Prestained Protein Standard Markers (lyophilized)	Freezer with desiccant	❑
B	Oligodendrocyte Proteins - (lyophilized)	Freezer with desiccant	❑
C	Neuron Proteins - (lyophilized)	Freezer with desiccant	❑
D	Astrocyte Proteins - (lyophilized)	Freezer with desiccant	❑
E	Microglia Proteins - (lyophilized)	Freezer with desiccant	❑
•	10x Tris-Glycine-SDS Buffer (Chamber Buffer)	Room Temperature	❑
•	FlashBlue™ Protein Stain Powder	Room Temperature	❑
•	Practice Gel Loading Solution	Room Temperature	❑
•	Transfer Pipets	Room Temperature	❑

There is enough of each sample for six (6) groups sharing three polyacrylamide gels.

Experiment Requirements *(NOT included with this experiment)*

- 12% Denaturing Polyacrylamide gels (3)
- Vertical Gel Electrophoresis Apparatus (Cat. #581 recommended)
- D.C. Power Supply
- Shaker Platform (optional)
- Automatic Pipettes with Tips
- Beakers
- Transfer Pipets
- Foil
- Spatula
- Latex or Vinyl Lab Gloves
- Safety Goggles
- Distilled Water
- White Vinegar
- Ethanol
- 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

The brain is an incredibly complex organ and is responsible for regulating almost everything within our body. It allows us to form complex thoughts, read, write, move, breathe, play sports, and listen to music. It does this through a network of cells working together to function.

All living things are made up of cells. They are the smallest unit of life, and the human body is composed of approximately 200 different types of cells. The brain is made up primarily of 4 types of cells: neurons, microglia, oligodendrocytes, and astrocytes (Figure 2). These cells work together to store information, keep the brain and body safe, and ensure that our bodies function properly.

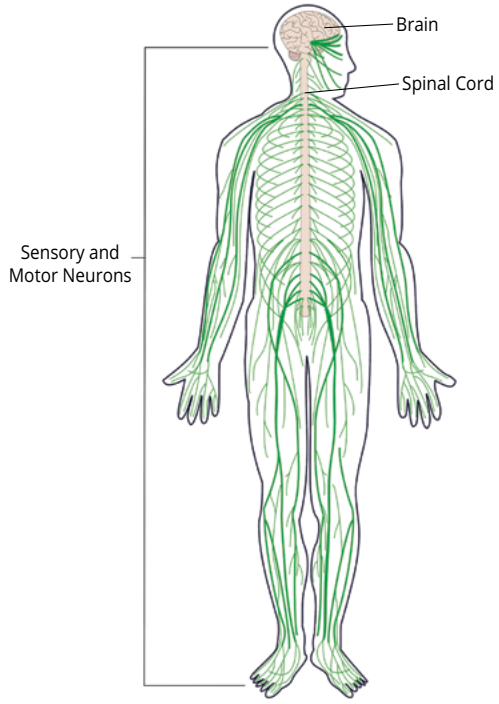


Figure 1: The Nervous System

Neurons represent the major source of information and communication within the brain. They send information through electrical impulses, and one neuron can communicate with 10,000 other neurons. Neurons are very large and complex cells. The average human cell has a diameter of 100 micrometers, but a neuron can be up to 1 meter long. That's 100,000 micrometers! Neurons are quite complex, as they must receive information, process it, and then pass it along to other neurons. They do this through having specialized areas within the cell (Figure 3). The cell body houses the nucleus, which contains all of the cellular information in the form of DNA. Surrounding the cell body are many dendrites, which receive information from neighboring neurons. When they receive enough input they fire an action potential, or a wave of electricity through the cell body and down the axon. Axons can be very long and transmit information very fast. When the action potential reaches the end of the axon, the neurons "fires", or releases small chemicals from the axon terminal. These small chemicals, or neurotransmitters, will bind to the dendrites of a neighboring neuron. Through this passage of electrical impulses and neurotransmitters, neurons are able to communicate with each other, store information, form memories, and control all of the functions within the body. While neurons are the primary cell type of the brain, they do not function alone. There are 3 other major cell types in the brain, and they are usually considered "support" cells. Research has shown that these cells also have very complex and critical functions within the brain.

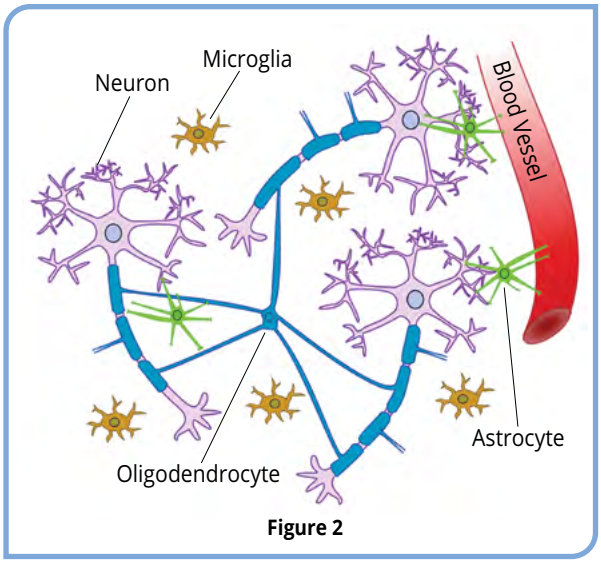


Figure 2

Oligodendrocytes are responsible for helping neurons communicate with each other. As mentioned above, neuronal axons can be very long, and they carry electrical signals. This is similar to how a wire can carry electricity. Most wires that we see are insulated with plastic or rubber. This insulation provides protection for the travelling signal. Axons are also insulated to ensure that they can communicate quickly. Oligodendrocytes wrap around a neuron's axon to provide this insulation. With this insulation, known as myelin, axons are able to transmit information at a rate of 150 m/second. Neurons without myelin are only able to transmit information at rates of around 1 m/second. Oligodendrocytes are critical to a neuron's

proper functioning, and problems with oligodendrocytes can be very bad for the brain. One major disease that targets oligodendrocytes is Multiple Sclerosis, which is caused by loss of myelin.

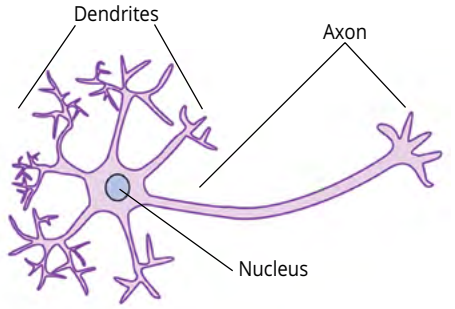


Figure 3: Areas of a Neuron

Microglia are the immune system of the brain. The immune system protects the body from infection, and microglia do the same thing in the brain. Microglia have been shown to be active during inflammation in the brain and are responsible for clearing up areas of dead cells or trash. Dysregulation of microglia has been shown to happen in diseases such as Alzheimer's Disease and AIDS.

Astrocytes are critical for regulating blood flow and nutrient retrieval in the brain. Neurons in the brain require a lot of energy and oxygen, which come from the blood. There are many blood vessels in the brain including major veins and arteries, as well as smaller capillary vessels. These blood vessels are wrapped by astrocytes to be sure that harmful cells and bacteria that may be traveling in the blood are kept out of the brain. Instead, astrocytes are able to regulate the diffusion of molecules from the blood into the brain so that neurons and other cells can use the oxygen and energy provided by the blood.

Each of these cell types plays a specific role in the proper functioning of the brain. However, it is impossible to differentiate them by their DNA code. Instead, the way that a DNA sequence is translated into protein, and which proteins are expressed within a cell, allows for each cell to have a unique profile and function. Every cell in a person's body contains the exact same DNA sequence (with the exception of the gametes, which only contain half). Therefore, in order to differentiate different cell types from the same organism, animal, or human, one must look at the profile of proteins that are expressed.

Similarly to how agarose gel electrophoresis can separate DNA fragments based on size, polyacrylamide gel electrophoresis can separate proteins based on size, shape, or charge. Unlike DNA, which always consists of nucleotides arranged in a double-helix structure, the amino acids of proteins can take on large and complex configurations. In order for these proteins to be accurately resolved by their size, they must first be denatured, or unraveled from their 3-dimensional structure.

Denatured proteins have lost their specific folding patterns and biological activity, but their amino acid chain remains in tact. In most cases, the proteins are denatured through boiling in the presence of sodium dodecyl sulfate (SDS) and 2-mercaptoethanol. SDS is a detergent consisting of a hydrocarbon chain bound to a negatively charged sulfate group. SDS binds to amino acids, giving the entire protein a net negative charge. Additionally, SDS binding causes proteins to unfold and helps in the denaturation process. However, even with SDS, some proteins have very strong bonds between amino acids, including covalent crosslinks known as disulfide bonds. These bonds are formed between two cysteine amino acid residues that can be located in the same or different polypeptide chains. High concentrations of reducing agents, such as 2-mercaptoethanol, will break disulfide bonds. This combination of SDS and 2-mercaptoethanol completely dissociates and denatures the protein.

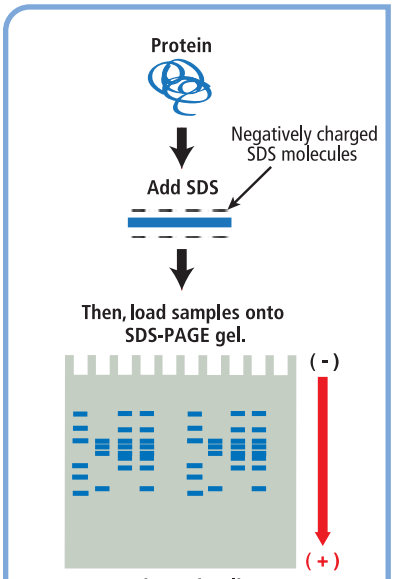


Figure 4

During electrophoresis, the SDS denatured proteins migrate through the gel towards the positive electrode at a rate that is inversely proportional to their molecular weight. In other words, the smaller the protein, the faster it migrates. This is because the polyacrylamide gel serves as a sort of maze for the proteins to travel through. The smaller the proteins, the easier and faster they transport through the gel. The molecular weight of an unknown protein is obtained by the comparison of its position on the gel after electrophoresis to the positions of a standard SDS protein ladder.

In this lab, you will be looking at the different cell types in the human brain by their expression of different proteins.

Experiment Overview

EXPERIMENT OBJECTIVE

The objective of the experiment is for students to examine the differences between cell types in the brain based on their profiles of proteins.

LABORATORY SAFETY

Wear gloves and safety goggles

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.

Acrylamide is a known neurotoxin and carcinogen and should be handled with extreme caution. Liquid acrylamide, used in the manufacture of SDS-PAGE gels, should only be handled in a chemical fume hood while wearing gloves and goggles. Polymerized acrylamide, including precast acrylamide gels, is safe but should still be handled with caution at all times.

LABORATORY NOTEBOOKS

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

Before starting the Experiment:

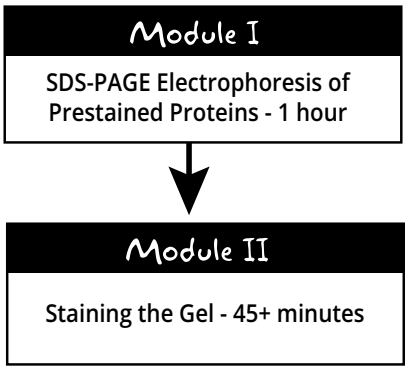
- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

During the Experiment:

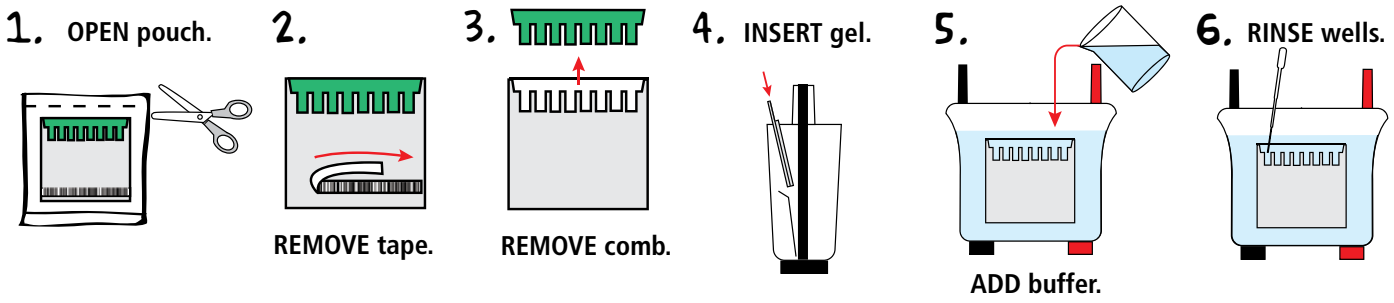
- Record (draw) your observations, or photograph the results.

After 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.



Module I: SDS-PAGE Electrophoresis of Prestained Proteins



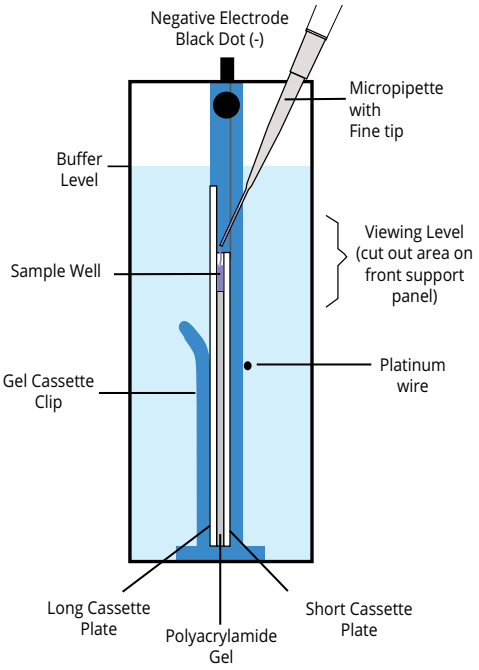
PREPARING THE POLYACRYLAMIDE GEL AND CHAMBER

Wear gloves and safety goggles

NOTE: Although precast polyacrylamide gels and protein chambers will vary slightly in design, the procedure for their use will be similar.

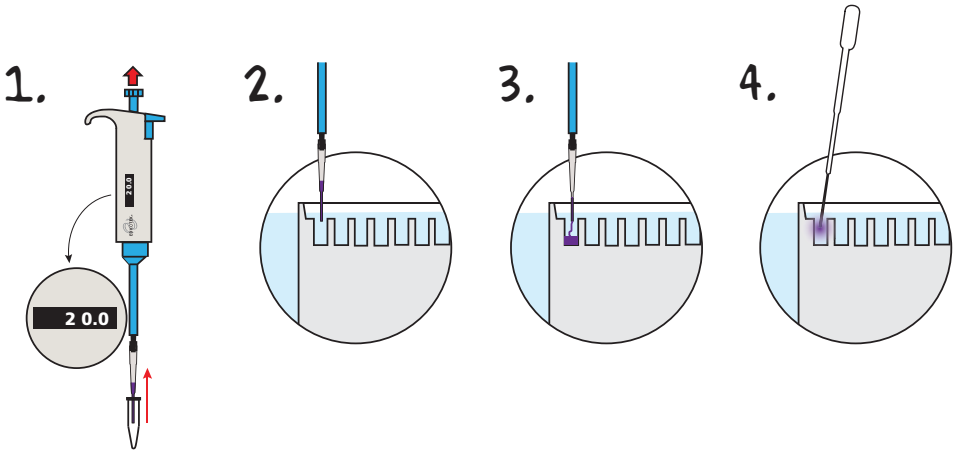
1. **OPEN** the pouch containing the gel cassette. Remove the cassette and place on bench with the shorter front plate facing up.
2. Gels may feature a sticker or tape at the bottom of the front plate. **REMOVE** the tape (if present) to expose the bottom of the gel.
3. Carefully **REMOVE** the comb by gently pulling upwards. Pull the comb straight up to prevent damage to the wells of the gel.
4. **INSERT** the gel into the electrophoresis chamber. Orient the gel according to the manufacturer's instructions. ***NOTE: For EDVOTEK® vertical electrophoresis chambers, the short plate should face the middle of the apparatus.***
5. **ADD** diluted electrophoresis buffer to the chamber. The buffer should cover the top of the shorter plate.
6. **RINSE** each well by squirting electrophoresis buffer into the wells using a transfer pipet. Using the transfer pipet, carefully straighten any wells which may have been distorted during comb removal or rinsing.

The gel is now ready for practice gel loading.



A polyacrylamide gel cassette in the EDVOTEK® Vertical Electrophoresis Apparatus, Model #MV10.

Module I: SDS-PAGE Electrophoresis of Prestained Proteins, continued



PRACTICE GEL LOADING (OPTIONAL)


Wear gloves and safety goggles

NOTE: EDVOTEK® Cat. #638, Fine Tip Micropipette Tips are recommended for loading samples into polyacrylamide gels. A regular microtip may damage the cassette and result in the loss of protein samples.

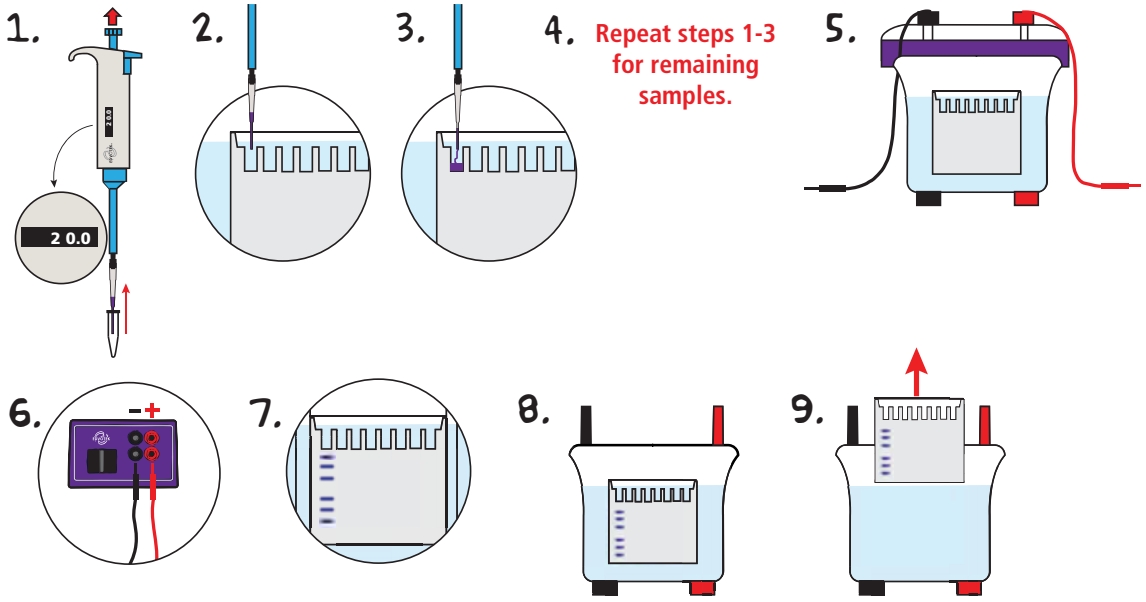
1. **PLACE** a fresh tip on the micropipette. **REMOVE** 20 µL of practice gel loading solution.

2. **PLACE** the lower portion of the pipette tip below the surface of the electrode buffer, directly over a sample well. The tip should be at an angle pointed towards the well. The tip should be partially against the back plate of the gel cassette, but the tip opening should be over the sample well. **Do not try to jam the pipette tip in between the plates of the gel cassette.**

3. **EJECT** all the sample by steadily pressing down on the plunger of the automatic pipette. Do not release the plunger before all the sample is ejected. Premature release of the plunger will cause buffer to mix with sample in the micropipette tip. Release the pipette plunger after the sample has been delivered and the pipette tip is out of the buffer.

4. **REMOVE** the practice gel loading solution from the sample wells. **FILL** a transfer pipet with buffer and **SQUIRT** a stream into the sample wells. This will displace the practice gel loading solution, which will be diluted into the buffer and will not interfere with the experiment. ***NOTE: Practice gel loading solution must be removed from the sample wells prior to sample loading.***

Module I: SDS-PAGE Electrophoresis of Prestained Proteins, continued



LOADING THE PROTEIN SAMPLES

Two student groups can share one gel. The first group should load samples into wells 1 - 5. The other group sharing the gel should load samples into wells 6 - 10 (see Table 1).

1. Using a fresh fine tip micropipet tip, **MEASURE** 20 µl of the first sample as indicated in Table 1. Sample A is the protein standard marker and should go in the first lane of your group's half of the gel.
2. **PLACE** the pipet tip under the buffer and directly above the sample well, resting gently against the back plate of the gel cassette.
3. Slowly **DISPENSE** the sample by depressing the plunger.
4. **REPEAT** steps 1-3 for remaining samples in Table 1, changing the tip between each new sample.

NOTE: Be sure to change pipet tips between loading each sample!

Table 1: Gel Loading

Lane	Sample
1	Protein Standard Markers (Group 1)
2	Oligodendrocyte Proteins (Group 1)
3	Neuron Proteins (Group 1)
4	Astrocyte Proteins (Group 1)
5	Microglia Proteins (Group 1)
6	Protein Standard Markers (Group 2)
7	Oligodendrocyte Proteins (Group 2)
8	Neuron Proteins (Group 2)
9	Astrocyte Proteins (Group 2)
10	Microglia Proteins (Group 2)

RUNNING THE GEL

5. Once all samples have been loaded, carefully **PLACE** the cover onto the electrode terminals.
6. **CONNECT** the electrical leads to the power supply.
7. **SET** the voltage of the power supply and **PERFORM** electrophoresis (See Table A for time and voltage guidelines). Allow the proteins to separate on the gel for the recommended length of time, or until the tracking dye reaches the bottom of the gel.

Note: When the current is flowing, you should see bubbles forming on the electrodes.

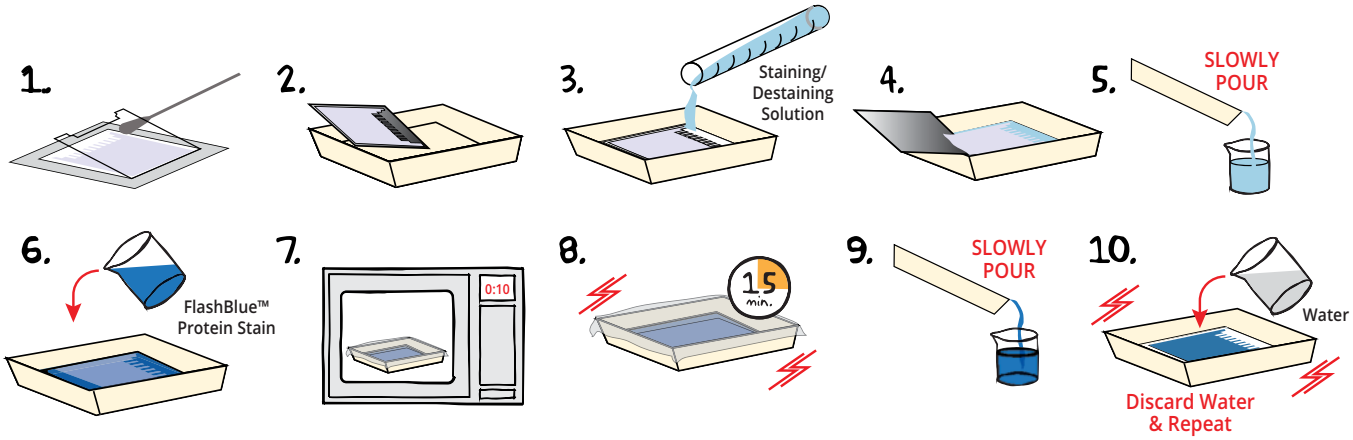
8. After the electrophoresis is finished, **TURN OFF** the power supply, disconnect the leads, and carefully **REMOVE** the cover.
9. **REMOVE** the gel cassette from the electrophoresis apparatus and immediately **PROCEED** to staining instructions on page 10.

Table A: Time and Voltage Guidelines

Volts	Recommended Time: Minimum	Recommended Time: Optimal
100	80 min.	95 min.
125	60 min.	75 min.
150	50 min.	60 min.

Module II: Gel Staining with FlashBlue™ Protein Stain

Although the protein samples are provided in a pre-stained format it is possible to increase the intensity of the bands by using FlashBlue™ Protein Stain. Staining is rapid and sensitive. Student groups that shared a polyacrylamide gel during electrophoresis should also stain this gel together.



1. After electrophoresis, **LAY** the cassette down and **REMOVE** the front plate by placing a thin spatula or screwdriver at the side edge and gently lift it away from the larger back plate. In most cases, the gel will stay on the back plate. If it partially pulls away with the front plate, let it fall onto the back plate. ***Handle very carefully as the thin gels are extremely fragile.***

2. **TRANSFER** the gel on the back plate to a clean tray.

3. **ADD** a sufficient volume (approximately 50-75 mL) of the staining/destaining solution into the tray to **COVER** the gel and back plate.

4. Carefully **REMOVE** the back plate from the tray, leaving just the gel in the tray containing the staining/destaining solution. Bands may be easier to see once the cassette is removed. **OBSERVE** the gel and take a photo/sketch the banding pattern in your notebook before continuing. ***NOTE: If the gel sticks to the plate, gently nudge the gel off the plate using two GLOVED fingers.***

5. **DISCARD** the staining/destaining solution. ***Pour slowly to keep the gel in the container.***

6. **ADD** 30 mL of prepared FlashBlue™ Protein Stain.

7. (OPTIONAL) **COVER** the container with plastic wrap and **MICROWAVE** for 10 seconds to gently heat the solution.

8. **INCUBATE** for 15 minutes at room temperature, **SHAKING** occasionally.

9. **DISCARD** the FlashBlue™ Protein Stain solution. ***Pour slowly to keep gel intact and in container.***

10. **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.

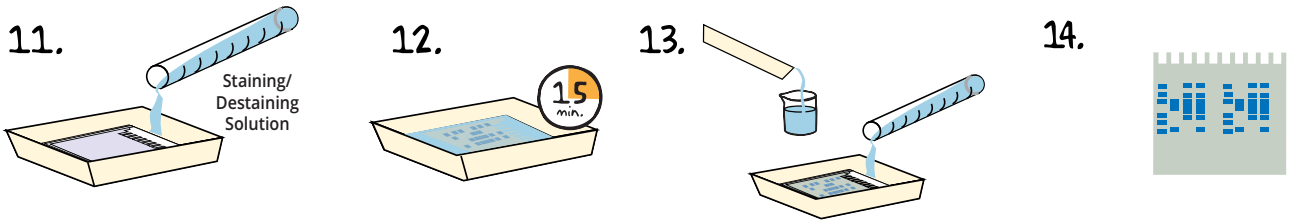
WEAR GLOVES AND SAFETY GOGGLES

Gloves must be worn during this procedure. Avoid touching the gel without gloves.

Polyacrylamide gels are very thin and fragile. Use care in handling to avoid tearing the gel.

continued

Module II: Gel Staining with FlashBlue™ Protein Stain, continued



11. **ADD** 30 mL of staining/destaining solution to the gel.

12. **INCUBATE** for 15 minutes at room temperature. **EXAMINE** the gel.

13. (OPTIONAL) **DISCARD** the used staining/destaining solution and **ADD** an additional 30 mL of staining/destaining solution. **INCUBATE** for 15-60 minutes at room temperature until the appearance and contrast of the protein bands against the background improves.

14. After staining, Protein bands will appear medium to dark blue against a light background* and will be ready for excellent photographic results.

** A white light box can be used to better visualize the protein bands.*

STORING THE GEL

- Gel may be left in deionized water for several hours with no loss in sensitivity and band intensity. This step should be performed once a desired background and stained protein bands are obtained. Pour off the destaining solution from Step 12 (or 13) and add a sufficient amount of deionized water to cover the gel.

- For permanent storage, the gel can be dried between two sheets of cellophane (saran wrap) stretched in an embroidery hoop. Air dry the gel for several days until the gel is paper thin. Cut the "extra" saran wrap surrounding the dried gel. Place the dried gel overnight between two heavy books to avoid curling. Tape it into a laboratory book.

Study Questions

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

1. What are the different cell types in the brain?
2. What do neurons do?
3. Why can we not determine the identity of a specific cell within a human based on its DNA?
4. How are proteins separated in an SDS-denaturing polyacrylamide gel?

Instructor's Guide

NOTES TO THE INSTRUCTOR

This experiment requires three 12% Polyacrylamide Gels to be shared by the 6 student groups. Each group requires 5 sample wells.

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances.

If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK® web site. In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

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

Pre-Lab Preparations

Specific Requirements for this Experiment

This experiment requires three 12% Denaturing Polyacrylamide Gels to be shared by the 6 student groups. Each group will require 5 sample wells. The protein standard marker (Component A) and Components B, C, D, and E must be reconstituted.

Protein Denaturation and Reconstitution of Lyophilized Proteins

1. Add 145 µl distilled water to each of the tubes, A-E. Vortex each tube for 30 seconds each, or until completely dissolved.
2. Wear safety goggles and bring a beaker of water, covered with aluminum foil, to a boil. Remove from heat.
3. Make sure the tube lids are securely fastened. Suspend the tubes in a boiling water bath for 10 minutes.
4. Remove and have students load gels while proteins are still warm. Tap or briefly centrifuge to get condensate at the top of the tubes back into the sample.

   This experiment contains practice gel loading solution. If you are unfamiliar with gel electrophoresis, it is suggested that you practice loading the sample wells before performing the actual experiment. Refer to instructions entitled "Practice Gel Loading Instructions".

IMPORTANT NOTE: Remember to use screw-cap tubes!

Tris-glycine-SDS Buffer (Electrophoresis Buffer Only)

1. Add 1 part EDVOTEK® 10X buffer to every 9 parts distilled water.
2. Make enough 1X buffer for the 3 electrophoresis units (2 liters for three EDVOTEK® units).

Electrophoresis Time and Voltage

Your time requirements will dictate the voltage and the length of time it will take for your samples to separate by electrophoresis. Approximate recommended times are listed in Table A.

Run the gel until the bromophenol blue tracking dye is near the bottom edge of the gel.

Table A — Time and Voltage Guidelines

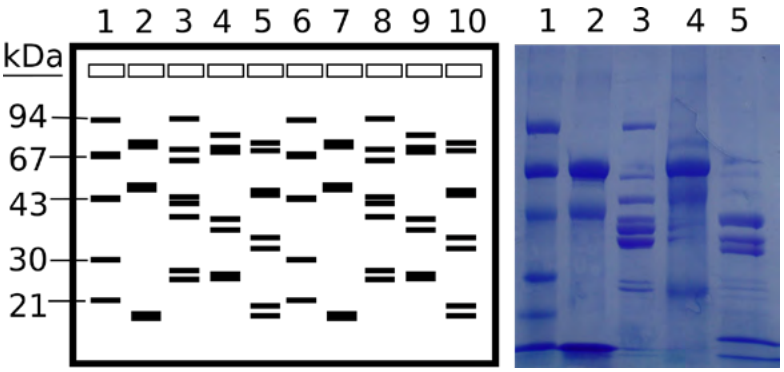
Volts	Recommended Time: Minimum	Recommended Time: Optimal
100	80 min.	95 min.
125	60 min.	75 min.
150	50 min.	60 min.

PREPARATION FOR STAINING GELS

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 130 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.
4. TWO student groups will share: 30 mL FlashBlue™ Protein Stain, 140 mL Staining/Destaining Solution, water, a staining tray, and plastic wrap.

**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.*

Expected Results and Analysis



Lanes	Contents	
1 and 6	A	Standard Protein Markers
2 and 7	B	Oligodendrocyte Proteins
3 and 8	C	Neuron Proteins
4 and 9	D	Astrocytes Proteins
5 and 10	E	Microglia Proteins

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

Appendix A
Troubleshooting Guide

SDS-GEL ELECTROPHORESIS TROUBLESHOOTING GUIDE		
PROBLEM:	**CAUSE:**	**ANSWER:**
Gel is not running properly.	Running buffer was not properly prepared.	Check buffer protocol, make fresh buffer.
	Wrong buffer used.	Check gel recipe, buffer must be compatible with the gel.
	Buffer volume is too low.	Buffer must fully cover the sample wells throughout the entire experiment.
	Gel is inserted in the wrong orientation.	Check with manufacturer for proper setup of the electrophoresis chamber.
	Malfunctioning electrophoresis chamber or power supply.	Consult with manufacturer of electrophoresis chamber or power supply.
	Tape at bottom of precast gel not removed.	Carefully remove tape before running the gel.
	Electrodes not connected or polarity reversed.	Check electrode connections at the gel box and power supply.
Poor band resolution or separation.	Diffusion of samples before power was turned on.	Minimize time between loading samples and the start of electrophoresis.
	The gel is old or expired.	Make fresh gels or order new pre-cast gels.
	Wrong concentration of polyacrylamide gel.	The kit is designed for 12% polyacrylamide gels, other concentrations will affect results.
Smiling or frowning of bands.	Proteins have been overloaded.	EDVOTEK® has optimized this kit to avoid overloading. Be sure to load the amount recommended by the protocol.
	Wrong buffer was used.	Check gel recipe, the buffer must be compatible with the gel.
	Incorrect voltage supplied to the gel.	Check the protocol for the recommended voltage.
No bands on gel/ smallest bands missing from gel.	Proteins ran off gel.	Use the appropriate length of time for the chosen voltage. Be sure to monitor the tracking dye while the gel is running. For best results, the tracking dye should run 8-9 cm and should not be allowed to run off the gel.
Proteins have accumulated in the wells of the gel.	Proteins have aggregated.	Ensure proteins have fully denatured; boil proteins for 5 min. and load while still warm.
Bands are smeary and distorted.	The gel has overheated.	Reduce voltage, check buffer concentration and dilute if necessary.
Bands are faint.	Proteins have diffused or faded.	Follow protocol for FlashBlue™ Protein Stain to increase the contrast of protein bands.
	Too little protein was loaded.	EDVOTEK® has optimized this kit to avoid underloading. Be sure to load the amount recommended by the protocol.