EDVOTEK® Quick Guide: Protein Electrophoresis

What is SDS-PAGE?

SDS polyacrylamide-gel electrophoresis, or SDS-PAGE, is a technique that is used to separate proteins according to their molecular weight.

What do I need to separate proteins?

In addition to your protein sample, you will need:

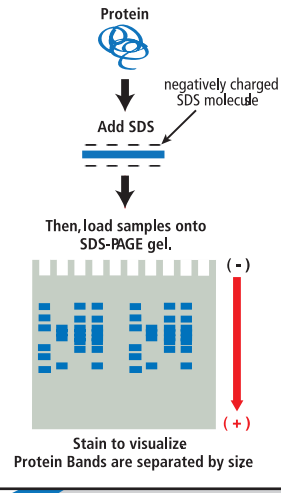
Sodium Dodecyl Sulfate (SDS) – a strong detergent with a hydrophobic tail and a negatively charged head.
Reducing agent - breaks covalent bonds between protein subunits.
Gel Loading Solution – includes glycerol to help protein samples enter into the wells and a visible dye to monitor sample migration through the gel.
Polyacrylamide gel - The separation matrix formed by polymerization of acrylamide monomers and chemical crosslinkers.
Electrophoresis Buffer – contains ions necessary to conduct an electrical current, maintains pH.
Vertical electrophoresis apparatus – holds the buffer and the gel, has positive and negative electrodes.
Power supply – generates the current necessary to move proteins through gel.
Micropipet and tips – used to transfer samples into wells.
Protein InstaStain™ -- used to visualize proteins

How does SDS-PAGE separate proteins?

Proteins produce a unique challenge for electrophoresis because they have complex shapes and different charges, which affect how they migrate through the gel. In order to accurately separate proteins by molecular weight and not by shape or charge, the secondary structure of the protein is unfolded using the anionic detergent sodium dodecyl sulfate (SDS) and a reducing agent. The SDS molecules form a complex with the protein, negating its inherent charge. The reducing agent breaks covalent bonds that link protein subunits.

After denaturation, the mixture of proteins is added into depressions (or "wells") within a gel, and then an electrical current is passed through the gel. Because the SDS-protein complex has a strong negative charge, the current drives the proteins through the gel towards the positive electrode. At first glance, a polyacrylamide gel appears to be a solid. On the molecular level, the gel contains channels through which the proteins can pass. Small proteins move through these holes easily, but large proteins have a more difficult time squeezing through the tunnels. Because molecules of different sizes travel at different speeds, they separate into discrete "bands" within the gel. After the current is stopped, the bands are visualized using a stain that sticks to proteins.

Figure 1: An Overview of SDS-PAGE



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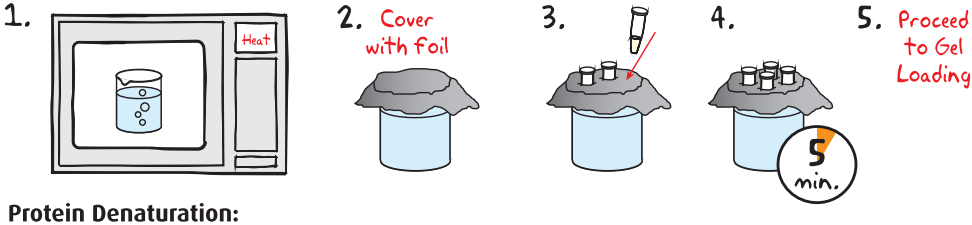
PERFORMING SDS-PAGE WITH PROTEIN SAMPLES

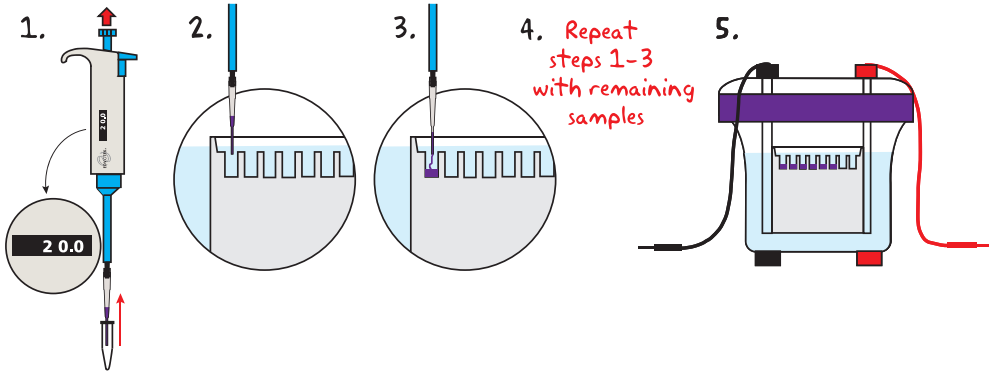


Protein Denaturation:

NOTE: PROCEED to gel loading if your lab instructor has already heated the protein samples.

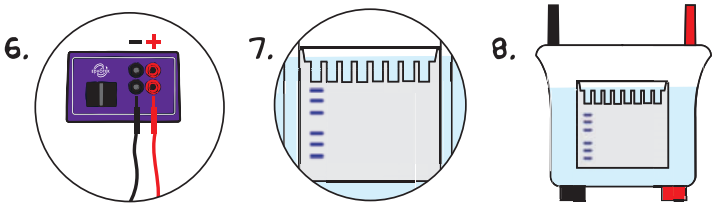
1. Using a hot plate or microwave, **HEAT** a beaker of water until it boils.
2. **COVER** with aluminum foil and carefully remove from heat.
3. Tightly **CAP** sample tubes. **PUSH** tubes through foil to suspend in the boiling water.
4. **INCUBATE** the samples for 5 minutes.
5. Immediately **PROCEED** to loading the gel while the samples are still warm.

FREEZING PROTEINS:
Unused portions of the protein samples can be frozen for later use. When needed, repeat steps 1-4 and proceed to Loading the Protein Samples.



Loading the Protein Samples:

1. Using a fresh pipet tip, **MEASURE** 20 µl of the Standard Protein Marker (A).
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 with remaining samples, changing the tip between each new sample.
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.
8. **TURN OFF** the power supply and carefully REMOVE the lid. The gel can now be removed from the chamber for staining.

The protein samples will need to be stained using Protein InstaStain® Cards (Cat # 2016). Staining is rapid and sensitive, and gels are ready for visualization in as short as 1 – 3 hours.

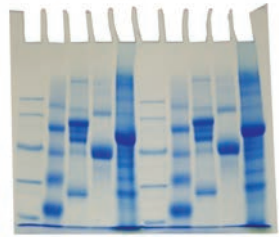
Table A Time and Voltage Guidelines

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

Related Product

Cat. #150
Survey of Protein Diversity

Learn about the diversity of proteins by studying the electrophoretic profiles of various sources. Your students will separate proteins from bacterial, plant, serum, and milk proteins alongside a standard protein marker.



Related Video - Protein Electrophoresis

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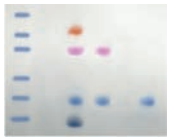
Cat. #151
AIDS Kit III: Simulation of HIV Detection by Protein Electrophoresis

NEW

The Human Immunodeficiency Virus (HIV) causes acquired immune deficiency syndrome (AIDS), a serious disease that suppresses a patient's immune system which leaves them susceptible to infections. In this experiment, students will use SDS-PAGE to simulate the identification of HIV proteins in simulated patient samples. The results of this test are used to diagnose an HIV infection.



Precast Polyacrylamide Gels
Cat. # 651
Three 12% precast gels.
9x10 cm. Requires refrigeration.

Cat. # 652
Six 12% precast gels
9x10 cm. Requires refrigeration.

Cat. #581
MV10 Vertical Protein Electrophoresis Apparatus

NEW

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Cat. #5010
TetraSource™ 300 30/300 V Power Supply

Power any combination of EDVOTEK electrophoresis units with this mighty 750 mA power supply! Features an easy-to-use, fully programmable interface for setting voltage, current or timer control with each parameter displayed in real-time. Programs may be paused or resumed at any point. Run experiments in the least time possible with this powerful and versatile unit! Made in the USA.

Cat. # 590
Edvotek® Variable Micropipet

This best selling Variable Micropipet is designed for volumes ranging from 5 to 50 µl. It is sturdy, easy to use, highly accurate and uses standard micropipet tips. The volume is selected by twisting the top. The lightweight design and tip ejector makes operation fast & easy. A tool and instructions are included for self-calibration.
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Protein InstaStain®

Protein InstaStain® sheets stain gels faster than conventional methods. Protein InstaStain® gives high quality and uniform gel staining with excellent results for photography. They are also environmentally friendly because they use a solid matrix, avoiding large amounts of liquid stain and waste disposal.

Cat. # 2016 For 15 gels, 7.5 x10 cm
Cat. # 2017 For 30 gels, 7.5 x10 cm

InstaStain® Protein