CHEG 4137
CHEMICAL ENGINEERING LABORATORY

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Background:

Gel electrophoresis is a method for separation of DNA, RNA, and proteins. It also provides a way to analyze samples based on size. When a voltage is applied to the gel loaded with the DNA, RNA, or protein samples, the molecules will migrate to the electrode of opposite charge. Shorter, smaller molecules move faster and further through the gel compared to larger molecules. In the case of proteins, the charge is dependent on the pH where the protein is found. However, this does not change the fundamental nature of electrophoresis; the proteins will migrate to the electrode of opposite charge.

In this experiment, protein samples will be loaded into a polyacrylamide gel for the electrophoresis procedure. This gel is prepared by mixing acrylamide monomers with a crosslinker as well as a catalyst. The concentration of acrylamide in the gel varies depending on the size of the proteins to be separated. The protein standard with known molecular weights (serving as the molecular weight markers) is run alongside the samples.

Proteins can have varying structures. To solve this conformation discrepancy, the proteins must be reduced and denatured. Therefore, protein samples are pretreated by heating them in the presence of the negatively charged detergent SDS, so that proteins and protein complexes are disrupted, or denatured, and all proteins acquire a uniform charge-to-mass ratio. Proteins then will migrate at rates dependent only on their molecular weights, without their native 3-dimensional shapes or charges being factors.

Safety Precautions:

1. ***Do NOT touch the electrophoresis chamber or wires when the voltage is turned on. Voltages may be as high as 300 V and shocks can be fatal. Do NOT remove the lid from the electrophoresis chamber until the power has been turned off. Before handling the electrophoresis apparatus, always make sure it is disconnected from the power supply.***
2. Acrylamide in the unpolymerized form is a skin irritant and a potential neurotoxin. Polymerized acrylamide should not present a safety hazard. However, as a precaution you are required to wear gloves when running this experiment.
3. Always wear proper PPE when running this experiment. This includes gloves, safety glasses, and a lab coat.
4. TEMED has a strong smell; do not leave the bottle open for longer than needed.
5. All polyacrylamide gel must be disposed of in the provided hazardous waste container

Procedure:

A basic procedure can be found below; each group will use this to develop their own detailed procedure for this experiment. The detailed procedure should include the necessary steps to create the hand-casted gel, run the protein samples, and analyze the results using the provided Image Lab software. Each group will also prepare a procedure for denaturing, separating, running, and analyzing the samples. Tutorials and other supporting documents are provided in this document, as well as on the ChegLabs website.

I. Handcasting Polyacrylamide Gels

1. Prepare the resolving and stacking gel solutions without APS or TEMED. Prepare

enough solutions to make 2 gels.

2. Clean the glass plates with ethanol and a kimwipe. Assemble the glass cassette apparatus. Remember to remove the green tape on the bottom, if necessary.
3. Place the comb in the assembled apparatus. Mark a spot on the glass plate approximately 1cm below the teeth of the comb; this will be the level to which the separating gel is poured. Remove the comb.
4. Add the APS and TEMED to the resolving gel solution, and pipet or shake to mix. Pour the solution to the mark. You may want to use a pipet.
5. Use a pipet to immediately overlay the solution with isopropanol or *n*-butanol.
6. Allow the gel to polymerize. Pour off the overlay solution and rinse the top of the gel with DI water three times.
7. Dry the area with filter paper or a kimwipe. Do not touch the gel with the paper.
8. Add the APS and TEMED to the stacking gel solution, and pipet to mix. Pour the solution on top of the resolving gel.
9. Insert the comb into the cell.
10. Allow the gel to polymerize, then remove the comb by slowly pulling it straight up.
11. Seal brown chemical bottles with parafilm before putting away.

II. Protein Sample Preparation

You will be provided with:

- S - the two standards in a 1:1 ratio (100uL)
- N – unknown protein (100uL)
- J – unknown protein (100 uL)
- U – unknown protein (100uL)

1. Label one microcentrifuge tube with the name of each protein sample being prepared for electrophoresis.
2. Add the necessary amount of Laemmli Sample buffer and β-mercaptoethanol (reducing agent) to each labeled tube.
    - The final concentration of protein should be ~0.5mg/mL
3. Heat the protein samples in Dry Bath for 5 minutes at 95°C to denature the proteins.
4. Store the samples on ice (short term) or in freezer (long term) until running SDS-PAGE.

III. Performing Electrophoresis

1. Prepare the running buffer.
2. Remove the comb from the gels and assemble the electrophoresis cell.
3. Fill the cell with buffer and check for leaks. Fill the cell up to above the small plate.
4. If there are no leaks, fill the inner and outer buffer chambers with running buffer. Use a pipette to remove air bubbles, if necessary.
5. Load the appropriate volume of the as-prepared protein samples to the gel. Load as quickly as possible to avoid diffusion.
6. Load protein standards to the gel.
7. Connect the electrophoresis cell to the power supply, then being the electrophoresis run.
8. After electrophoresis is complete, turn off the power supply and disconnect the leads. Open the gel cassettes and carefully remove the gel.

IV. Imaging

The imaging software is available on the ChegLabs website.

1. Place a small amount of buffer solution onto the tray.
2. Wet your gloved hands with DI water. Pick up the gel from the bottom of the gel, then carefully place the gel on the provided imaging tray. Try to avoid air bubbles. Place this tray into the imager and take the image.
3. After the image has been captured, use the "Volume Tools" analysis tool to draw rectangles around each band to be analyzed. The bands from the standards can be set as such by double-clicking on the boxes.
4. The "Lanes and Bands" tool can be used to analyze lanes, or to detect bands. Manual selection is more accurate than automatic selection.

Available Consumables:

- Two unknown protein solutions
- 10X Tris/Glycine/SDS
- Ammonium Persulfate (APS)
- TEMED
- Laemmli sample buffer
- 2-Mercaptoethanol (reducing agent)
- Protein All Blue Standards
- Protein Unstained Standards
- TGX Stain-Free Acrylamide Kit
    - FastCast Stacker A
    - FastCast Stacker B
    - FastCast Resolver A
    - FastCast Resolver B

Documentation:

Making the gel:
https://www.youtube.com/watch?v=EDi_n_0NiF4
https://www.youtube.com/watch?v=XUjLO-ek2C8
A Guide to Polyacrylamide Gel Electrophoresis
Handcasting Polyacrylamide gels

Imaging:
https://www.youtube.com/watch?v=h6Cjy9dW95g
https://www.youtube.com/watch?v=dcCMPVjDPVs
https://www.youtube.com/watch?v=NRcq5pVTdH4
https://www.youtube.com/watch?v=DNQVfWYKcOo
https://www.youtube.com/watch?v=JiV51QfrvPU

Introduction to electrophoresis:
Select the "Protein Electrophoresis" presentation at this link:
http://www.bio-rad.com/en-us/applications-technologies/presentations-activities-for-workshops-teaching
http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6040.pdf