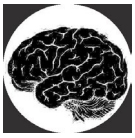
	SKILLS CENTER **STANDARD OPERATING PROCEDURE**	**A BIOFIZZ**  **PRODUCTON**
SDS-PAGE **Protein Electrophoresis** **Module Hours: 4**	**Effective Date: 10/14/2021** **PRQs** **Pipette calibration** **Buffers, stock solutions, and dilutions** **Centrifugation**	**Revision # 1.0** **M. Guzie** **Checked:** **M. Stowell**

BACKGROUND

Sodium Dodecyl Sulfate Poly Acrylamide Gel Electrophoresis, or SDS-PAGE, is a common molecular technique used to separate proteins by molecular weight. It is a useful method to separate samples which may contain many different proteins. The first part of the acronym, SDS, refers to the detergent Sodium Dodecyl Sulfate treatment which proteins are treated with before undergoing electrophoresis. Proteins are boiled in sodium dodecyl sulfate, which denatures the proteins and distributes a uniform charge across the structure (Stephen, 2021). The purpose of the procedure is for the proteins to be separated based solely on molecular weight, so it is important for the secondary and tertiary structures of the protein to be denatured so every protein has the same uniform linear shape as it travels through the polyacrylamide gel matrix. It is also important for a uniform charge to be distributed across the protein, so each protein is being pulled to the positive end of the gel with the same force.

The second part of the acronym, PAGE, stands for Poly Acrylamide Gel Electrophoresis. A polyacrylamide gel is used as the vehicle, and a charge is applied to the gel chamber so that the negatively charged proteins will be drawn from the anode (negatively charged end of the gel chamber) to the cathode (positively charged end of the gel chamber.) They migrate through the polyacrylamide matrix, drawn to the positive charge; smaller proteins will migrate faster through the gel, while the larger proteins will have a harder time reaching the cathode. This allows for the proteins to be separated by molecular weight (Stephen, 2021).

The methodology of SDS-PAGE became popular in cell biology very quickly after a MIT grad student discovered the ability of SDS to denature viral proteins of *E. Coli*, which allowed for further study of the virus. The student found that when SDS was included in protein samples during electrophoresis, the protein's ability to be separated through electrophoresis were greatly enhanced (Thoru, 2008). Protein purification and separation techniques have evolved greatly from early primitive methods to an era now where the sophisticated, efficient separation of proteins (and other macromolecules, see DNA Gel Agarose SOP) is commonplace in most all biology laboratories. This module will describe how to set up and run an SDS-PAGE protein separation technique.

1. PURPOSE

The purpose of this procedure is to understand the basic principles of the protein separation process known as SDS-PAGE and to be able to conduct the process in the lab.

2. SCOPE

This procedure applies to qualified skills center users.

3. RESPONSIBILITY

3.1. It is the responsibility of the user to understand and perform the procedure described in this document.

3.2. It is the responsibility of the user performing the procedure to fully document any deviations from the written procedure.

3.3. It is the responsibility of the user to become trained on the procedure.

4. DEFINITIONS

4.1. Antioxidant: Chemical substances which protect from oxidation; oxidation can be harmful to living cells and create reactive species such as free radicals.

4.2. Reducing Agent: Chemical substances which act in opposition to oxidants or oxidizing agents; rather than taking away electron density, reducing agents donate electron density to substrates.

4.3. Gel Electrophoresis: A common laboratory technique in molecular biology which utilizes an agarose gel to separate macromolecules (nucleic acids and proteins) by size using an electrode to pull molecules through the gel.

4.4. Molecular Weight Markers: A set of standard proteins of known sizes which are used to compare experimental results against and determine molecular weight.

4.5. Denature: When proteins (or nucleic acids) lose all quaternary, tertiary, and secondary structural characteristics due to other molecular forces or energy

(i.e. pH, heat) interfering with the forces which usually keep the molecule in its three-dimensional shape.

5. MATERIALS/EQUIPMENT

5.1. Polyacrylamide Gel (pre-made; remove green strip on bottom before running)
5.2. Gel chamber & power supply
5.3. Micropipettes
5.4. Disposable pipette tips
5.5. Protein samples
5.6. Molecular weight marker (Sigma MW-SDS-Blue.)
5.7. Running buffer (contains SDS)
5.8. Sample buffer
5.9. Graduated cylinders
5.10. Microcentrifuge tubes
5.11. Microcentrifuge
5.12. Hot plate shaker (used for both heating and shaking)
5.13. Staining trays
5.14. RAPIDstain solution
5.15. Syproruby stain
5.16. Syproruby – Fixing solution
5.17. Syproruby – Wash solution

6. RECIPES

6.1. Running Buffer (10X; 1L:)
(Ref: http://cshprotocols.cshlp.org/content/2006/1/pdb.rec10475.full)
Dissolve 30.0 g of Tris base, 144.0 g of glycine, and 10.0 g of SDS in 1000 ml of H2O. The pH of the buffer should be 8.3 and no pH adjustment is required. Store the running buffer at room temperature and dilute to 1X before use.

6.2. Syproruby – Fixing solution (1X; 500 ml)
50% Methanol (MeOH) - 250 ml MeOH
7% Glacial acetic acid – 35 ml Acetic acid

Up to 500 ml with DI H20

6.3.Syproruby – Wash solution (1X; 250 ml)
10% MeOH – 25 ml
7% Glacial acetic acid – 17.5 ml
Up to 250 ml with DI H20

7. PROCEDURE

The SDS-PAGE procedure will begin with preparation of protein samples, and then proceed with running the electrophoresis; the poly acrylamide gel will be already prepared in the lab.

7.1. **Preparing Protein Samples (SDS Treatment)**

7.1.1. Begin by treating all protein samples with the sample buffer (which includes SDS.) For each protein sample, put the following contents into a sterile microcentrifuge tube:
-25 µL 2x Sample Buffer
-25 µL protein sample

7.1.2. Mix gently with pipette (aspirate/dispense 3x.)

7.1.3. Heat the sample to 95 °C for five minutes.

7.1.4. After heating the sample, pulse samples in the microcentrifuge for approximately 30 seconds.

7.2. **Preparing Gel Box**

7.2.1. The gel box will already be assembled. Place the electrode chamber in the middle of the gel box. Place pre-made poly acrylamide gel in the electrode chamber. Make sure the other side of the electrode chamber is blocked by a blocking wall, or another gel if you are running two gels. This will make the interior chamber closed in. (NOTE: If using premade gels, be sure to **remove**

the small green strip of tape at the bottom of the gel prior to assembly into the electrode chamber)

7.2.2. Make sure the electrode chamber is sealed tightly by filling it up with running buffer and making sure there are no leaks.

7.2.3. Add 200 mL (or enough to submerge wells) of the SDS running buffer into the interior chamber.

7.2.4. Add 600 mL (or about enough to fill ¼ of the exterior chamber) of SDS running buffer into the larger exterior buffer chamber. (outside of electrode chamber).

7.3. **Loading Protein Samples**

7.3.1. Gently remove comb from polyacrylamide gel to reveal wells.

7.3.2. After the samples have cooled down to room temperature, begin adding them to the wells of the gel.

7.3.3. Use a micropipette and *gel loading tips* (new tips each time) to load volume of each sample into their own well.

7.3.3.1. Check the box from which you got the gel to determine well capacity. Boxes will say anywhere from 15uL to 30uL. Load about 3uL **less** than the gel box says.

7.3.4. Load 2 µL of the molecular weight marker into one well (usually either first or last well on the ends of gel.)

7.3.5. Create a table identifying each well with sample added and amount (will be submitted with module mastery task; can be virtual or physical.)

7.4. **Running the Electrophoresis**

7.4.1. Once the gel has been fully loaded, plug in the gel chamber to power supply.

7.4.2. Run the gel at 150 V for 40-60 minutes.

7.4.3. Turn off the power supply when the dye is nearly at the bottom of the gel (about 1 cm.) The dye is smaller than the proteins so it will reach the bottom of the gel before the proteins.

7.5. **Staining the Gel**

7.5.1. Stain the protein gel to have a document for analysis; begin by taking apart the gel box and then removing the gel.
7.5.2. Clean the gel box. Rinse with DI water, making sure not to get the top of the gel box and electrical components wet.
7.5.3. Place the protein gel in the staining tray.
7.5.4. Wash the gel 3 times with DI water (approximately 5 minutes on shaker each time.)
7.5.5. Add fixative solution until the gel is completely submerged; place on shaker for 15 minutes.
7.5.6. Discard fixative solution into hazardous waste disposal.
7.5.7. Repeat 7.5.4. (another DI wash.)
7.5.8. Add approximately 50 mL of stain and allow to stain for 1-24 hours, depending on protein content, placed on shaker.
7.5.9. After allowed to stain, decant stain into hazardous waste.
7.5.10. Wash gel with DI water again, placed on shaker for 15 minutes; depending on protein/stain content, may take more time.
7.5.11. Remove the gel from the staining tray and place on the visible light box so a photo can be taken and results can be analyzed (see module mastery task, 10.)

8. TROUBLE SHOOTING

8.1 If the upper buffer chamber does not stay full check that you have assembled the dummy gel correctly so that it makes a proper seal.
8.2 If bands are not sharp and form a smiley, check that you have removed the bottom gel tape.

9. REFERENCES

Audino, D. (2008, April 4). *SDS-PAGE SOP*. Great Bay Community College, Portsmouth, NH. Retrieved from http://biomanufacturing.org/uploads/files/74523064768154312-sds-page-sop.pdf.

Ricardo, S. (2021). *Chapter 4: Protein Structure*. B CHM 4611. Protein Structure (Web view).

Thoru, P. (2008, April 1). *Turning a page: The overnight sensation of SDS-polyacrylamide gel electrophoresis*. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. Retrieved from https://pubmed.ncbi.nlm.nih.gov/18378803/.

10. MODULE MASTERY TASK

For this task you will analyze 3 protein samples by SDS page and use the observed MW to identify the protein samples. The 5 available protein samples available are human hemoglobin, horse heart cytochrome C, porcine insulin, ribonuclease A from bovine pancreas and bovine albumin

10.1. Choose any 3 protein aliquots (A, B, C, D, or E) from the SDS-PAGE tray in the –20 freezer. Each sample is a different protein at a concentration of 1mg/mL.

> Each of the five unknowns were prepared from the following (Randomized):

- Hemoglobin
- Ribonuclease A
- Bovine Serum Albumin
- Cytochrome C
- Insulin, Porcine

10.2. Prepare each protein sample separately as per the SOP

10.3. Obtain a premade acrylamide gel and assemble the gel electrophoresis device with the premade gel.

10.4. You will be running your set of four samples (marker and 3 unknowns) twice on the same gel. After electrophoresis is completed you will be cutting the gel in half and staining each half using a different protein staining technique.
Run samples on opposite ends of the gel (Ex. Use lanes 1-4 & 7-10).

For your first set of four samples, use *gel loading tips* to load 10µl of a MW marker and 40µl of each of your 3 chosen unknowns into the first four wells.
This set will be stained using the RAPIDstain method.

Dilute the second set of four samples 10-fold prior to loading in second half of the gel. These will be stained using the SyproRuby protien staining protocol. This is a far more sensitive stain than RAPIDstain and we'll get to see how.

10.5. Run the SDS-PAGE gel as per the SOP.

10.6. Remove the plastic plates from the gel and carefully cut the gel in half vertically between the two sets of samples.

10.7. Stain the first set of samples (first half of gel; 1X concentration samples) in a staining box via RAPIDstain (use the directions seen on the bottle or found here: https://cdn.gbiosciences.com/pdfs/protocol/786-31_protocol.pdf).

10.8. Stain the second set of samples (second half of gel; 1/10X concentration samples) in a separate staining box via Syproruby (Directions on the web: https://assets.thermofisher.com/TFS-Assets/LSG/manuals/mp12000.pdf.). You can choose to run the basic or the rapid protocol, just indicate which you used.

10.9. After staining, take a photo of your gel on the light box for the RAPIDstain samples and the UV box for the syproruby samples.

10.10. Submit your photo with an analysis of your results. Using the molecular weight bands as comparison, how large are each of the sample proteins? Can you deduce what protein is in each sample based on the observed molecular weight and the known molecular weight of the 5 protein samples? Give a descriptive analysis of what information can be drawn about each of the proteins, if the results seem accurate with what wells the sample proteins were placed in,

and if there seems to be any indication of contamination or any discrepancies in observed and expected protein size.

10.11. Include the chart (7.3.5.) with your submission that specifies which samples are in which wells. Before submission, add a row/column to the chart which indicates the estimated molecular weight of each protein.

10.12. Compare your two protein staining methods. Which provided clearer results? What value is there in each of these protein staining methods?