

EXERCISE 8C

Name _____

Isolating, purifying, and characterizing proteins

Day Three: *How can gel electrophoresis be used to separate the proteins present in your milk fractions?*

Objectives

After completing this exercise, you should be able to:

- ◆ Describe and use discontinuous Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) to separate proteins in a mixture.

Prelab

Before you come to lab, read this entire exercise. You must also answer all questions and complete all assignments on the first 6 pages of this exercise. Your instructor will give you directions on when and where to turn in your work.

During Lab 8A, you attempted to isolate α -lactalbumin from the other molecules found in nonfat milk. At several points during the procedure, you saved samples of the milk fractions. Today, these fractions will be analyzed using SDS-PAGE electrophoresis in order to assess the effectiveness of the various purification steps.

Electrophoresis is a powerful technique that can be used to separate a mixture of molecules into its components. During electrophoresis, the molecules to be separated are loaded onto a support medium and then subjected to an electrical field. The **cathode** (negative electrode) attracts positively charged molecules, while the **anode** (positive electrode) attracts negatively charged molecules. This attraction causes the molecules in the mixture to migrate through the support medium at different rates depending on their size, shape, chemical composition, and electrical charge. As the molecules migrate at different rates, they gradually separate from each other.

All types of electrophoresis separate molecules based on the principle just described. Different types of electrophoresis vary mainly with respect to the type of support medium used, the buffer system used, and how the samples are prepared. One type of electrophoresis commonly used to separate proteins is **discontinuous Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)**.

In PAGE, a polyacrylamide gel functions as the support medium. Polyacrylamide gels are formed by polymerization of **acrylamide** with the cross-linking agent **bis-acrylamide**. The pore size of the gel can be adjusted by varying the concentrations of acrylamide and bis-acrylamide. The lower the concentrations of acrylamide and bis-acrylamide in the gel, the larger the pore size will be. Pore size affects what size proteins can travel through the gel and how fast they will travel. The larger the pore size, the faster the proteins will migrate.

An improvement in the resolution of polyacrylamide gels can be obtained by allowing the proteins to migrate through two gel layers instead of one. With the gel oriented vertically, first the proteins migrate through an upper **stacking gel**, and then they migrate through a lower **resolving gel**. The buffer used to prepare the stacking gel has a lower concentration of mobile ions than the buffer used to prepare the resolving gel. Therefore, when the proteins migrate through the stacking gel they serve as the principle charge carriers and migrate faster. On the other hand, when the proteins migrate through the resolving gel, which has a higher concentration of other mobile ions, they migrate slower. In addition, the stacking gel has larger pores than the resolving gel. This also allows the proteins to migrate more rapidly through the stacking gel. As a result, during electrophoresis the proteins move very rapidly through the stacking gel, and then suddenly slow down and “stack up” in a very thin band when they reach the resolving gel. Therefore, the proteins all begin a slower migration through the resolving gel from the same sharp band. This produces sharp, distinct bands in the resolving gel, providing good resolution of the different proteins.

The movement of proteins through a polyacrylamide gel is potentially influenced by many factors. Small proteins will move through the gel more quickly than larger ones. Proteins containing more amino acids with negatively charged R-groups will move toward the positive anode more quickly than proteins with fewer of these amino acids. Proteins folded into tight, compact shapes will move through the gel more quickly than bulkier proteins. The influence of so many confounding factors would make it difficult to deduce the qualities of the proteins that have been separated by PAGE. For example, which protein would reach the anode first—a small protein with fewer negative charges, a larger one with more negative charges, or a compactly folded protein that is larger than the first but has more negative charges than the second?

In order to reduce the number of factors that influence the migration rate of proteins through a polyacrylamide gel, the protein sample is treated with a reducing agent, such as **mercaptoethanol** and a detergent, usually **sodium dodecyl sulfate (SDS)**. The mercaptoethanol breaks the disulfide bonds that help stabilize the tertiary structure of a protein. The SDS further disrupts the three-dimensional structure of the protein by binding to the hydrophobic regions of the protein, covering them with negative charges. This causes the denaturation, or unfolding, of the protein. Finally, the sample is heated to 90-100° C to insure complete denaturation. What remains is a long string of amino acids, coated with negatively-charged SDS molecules.

When the SDS coated proteins travel through the polyacrylamide gel, their rate of movement toward the anode is determined almost entirely by size alone. Under these conditions, there is a linear relationship between the log of the molecular weight of the protein and its migration distance. Because of this, SDS-PAGE can be used to estimate the molecular weight of a protein. In order to do this, a standard curve that shows the relationship between the migration distance through the gel and the log of the molecular weight must be generated. The standard curve is generated by measuring the migration distance of several proteins of known molecular weight, plotting a scatter diagram of migration distance vs. log of MW, and then using linear regression to determine the equation of the “best fit” straight line for the data points. Once this is done, you can substitute the migration distance of any protein on your gel into the linear regression equation and then calculate the log of MW for that protein.

Main steps in purifying & analyzing α -lactalbumin

1. Set aside a sample of nonfat milk to assay later
2. Precipitate the casein milk proteins using heat and low pH. The other milk proteins should remain in solution. Centrifuge the heat and acid treated milk to separate the precipitated casein proteins from the soluble proteins.
3. Set aside a sample of the pellet of precipitated proteins to assay later.
4. Remove any remaining precipitated proteins from the supernatant (whey) using ultrafiltration.
5. Set aside a sample of the whey to assay later.
6. Separate α -lactalbumin from the other proteins that remain in the whey using size exclusion chromatography.
7. Set aside the five chromatography fractions most likely to contain α -lactalbumin to assay later.
8. Prepare a standard curve for the Bradford assay using solutions of known protein concentration.
9. Use the Bradford assay and your standard curve to determine the protein concentrations of the 8 milk fractions that you set aside on Day 1.
- 10. Calculate the amount of each milk fraction that should be loaded into your SDS-PAGE gel so that each lane contains an appropriate amount of protein.**
- 11. Load, run, and stain SDS-PAGE gels.**
12. Analyze your gel to determine the number of different proteins that are present in each milk fraction and the molecular weights of these proteins.
13. Use your gel analysis to evaluate how effectively you isolated and purified α -lactalbumin from the other components of milk.

Report the protein concentration of your diluted milk fractions

During Lab 8B, you used the Bradford assay to determine the protein concentrations of your **diluted** milk fractions. Report your results in the table below. Report the A_{595} values for all 11 diluted milk fractions, but report protein concentrations only for the diluted fractions whose A_{595} value fell within the linear range of your standard curve. If there are any diluted fractions whose A_{595} value fell outside the linear range of your standard curve, simply write “NA” in the column labeled “Protein concentration.”

Protein Concentration of Diluted Milk Fractions		
Milk fraction (dilution)	A595 value	Protein concentration ($\mu\text{g}/\mu\text{L}$)
Nonfat milk (2.0 %)		
Nonfat milk (3.0 %)		
Pellet (3.0 %)		
Pellet (5.0 %)		
Whey (50.0 %)		
Whey (100.0%)		
Column fraction ____ (100 %)		
Column fraction ____ (100 %)		
Column fraction ____ (100 %)		
Column fraction ____ (100 %)		
Column fraction ____ (100 %)		

Report the protein concentration of your undiluted milk fractions

Use the table below to report the protein concentrations of your **undiluted** milk fractions. As explained in Lab 8B, you can calculate the protein concentration of each **undiluted** milk fraction by taking the protein concentration of the corresponding **diluted** milk fraction and dividing it by the dilution percent. For example, if a 1% dilution of nonfat milk had a protein concentration of 380 $\mu\text{g}/\text{mL}$, then to calculate the protein concentration of the **undiluted** nonfat milk simply divide 380 $\mu\text{g}/\text{mL}$ by 1% (0.01). This means the undiluted nonfat milk had a protein concentration of 38,000 $\mu\text{g}/\text{mL}$ or 38 mg/mL .

NOTE: When performing the Bradford assay, you used 2 different dilutions of 3 milk fractions: the nonfat milk, the pellet, and the whey. In each case, you should calculate protein concentrations using only the dilution that produced an A595 value that lies within the linear region of your standard curve. If **both** dilutions of a given milk fraction gave an A595 value that lies within the linear region of your standard curve, then calculate the protein concentration of both diluted fractions, use both results to calculate the protein concentration of the undiluted milk fraction, and then average the 2 answers.

Protein Concentration of Undiluted Milk Fractions	
Sample Type	Protein concentration ($\mu\text{g}/\mu\text{L}$)
Nonfat milk	
Pellet	
Whey	
Column fraction ____	
Column fraction ____	
Column fraction ____	
Column fraction ____	
Column fraction ____	

Calculate the protein concentration of your undiluted milk fractions after the 2X sample treatment buffer was added

During Lab 8B, you prepared your undiluted milk fractions for electrophoresis by mixing 50 μ L of each undiluted milk fraction with 50 μ L of 2X sample treatment buffer. This effectively dilutes the protein concentration of each sample in half. Taking this into account, calculate the protein concentration of each undiluted milk fraction after the 2X sample treatment buffer was added, and report the results in the table below:

Protein Concentration of Undiluted Milk Fractions After the 2X Sample Treatment Buffer was Added	
Sample Type	Protein concentration (μg/μL)
Nonfat milk	
Pellet	
Whey	
Column fraction ____	
Column fraction ____	
Column fraction ____	
Column fraction ____	
Column fraction ____	

Calculate dilutions for any milk fraction containing 2X sample treatment buffer that have a protein concentration greater than 1.25 μ g/ μ L

It is important to load an appropriate amount of protein into each well of your electrophoresis gel. If too little protein is loaded, it may be difficult or impossible to see many of the protein bands after the gel is stained. On the other hand, if too much protein is loaded, the bands may become too dark and wide causing them to overlap and form a smear. In general, the optimum amount of protein to load into each well is about **20 μ g**.

Also keep in mind that there is a limit to the volume of sample that can fit into each well on your gel. To avoid overfilling the wells, it is best to load about **16 μ L** of sample into each well. This means that, ideally, **16 μ L** of sample should contain **20 μ g** of protein. Therefore, each sample that you load onto your gel should have a protein concentration of **20 μ g/16 μ L, or 1.25 μ g/ μ L**. This is the desired protein concentration because if you load **16 μ L** of sample into a well, and your sample has a protein concentration of **1.25 μ g/ μ L**, then the well will have **20 μ g** of protein (**1.25 μ g/ μ L X 16 μ L**), which is the ideal amount.

With this in mind, examine the actual protein concentrations of the undiluted milk fractions containing 2X sample treatment buffer, which are listed in the table directly above. There are 3 possible cases:

- 1) The protein concentration equals **1.25 μ g/ μ L**. In this case, the sample has the desired protein concentration. Simply load 16 μ L of the milk fraction into the well, and the well will contain the optimum amount of protein (20 μ g.)
- 2) The protein concentration is less than **1.25 μ g/ μ L**. In this case, the protein concentration is lower than desired. Unfortunately, there is no easy way to increase the protein concentration of your sample. The best you can do is load the maximum amount of sample that the well can hold (16 μ L). Keep in mind that your well will have less than the optimum amount of protein, meaning some of the protein bands on your gel may be very light or invisible after staining.

- 3) The protein concentration is greater than **1.25 µg/µL**. In this case, the milk fraction should be diluted with 1X sample treatment buffer to produce a sample with a protein concentration equal to **1.25 µg/µL**. For this dilution, calculate how much of the milk fraction with 2X sample treatment buffer and how much 1X sample treatment buffer should be combined to make **40 µL** of sample with a final protein concentration of 1.25 µg/µL. After you have prepared **40 µL** of the diluted sample (containing 1.25 µg/µL of protein), you will load **16 µL** of this sample into the well and discard the remaining 24 µL.

Fill in the table below (and the table on p. 8 of the Procedures section) to show how you will dilute the milk fractions containing 2X sample treatment buffer. In each case, your diluting solution will be 1X sample treatment buffer. The final volume of your diluted sample should be 40 µL and the final protein concentration should be 1.25 µg/µL. For those milk fractions containing 2X sample treatment buffer that already have a protein concentration of 1.25 µg/µL or less, simply write “no dilution necessary”:

Dilution of Milk Fractions to Make 40 µL of Diluted Sample with a Protein Concentration of 1.25 µg/µL		
Milk fraction	Amount of milk fraction with 2X sample treatment buffer needed (µL)	Amount of 1X sample treatment buffer needed (µL)
Skim milk		
Pellet		
Whey		
Column fraction		
Column fraction		
Column fraction		
Column fraction		
Column fraction		

Lab Procedures:

SAFETY WARNING: Acrylamide in the unpolymerized form is a skin irritant and a potential neurotoxin. Fortunately, the acrylamide in your gels is polymerized, so it should not present a safety hazard. However, as a precaution you are required to wear gloves when handling gels, buffers, stains, and destaining solutions.

SAFETY WARNING: 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. Before handling the electrophoresis apparatus, always make sure it is disconnected from the power supply.

I. Prepare the electrophoresis apparatus for SDS-PAGE

1. Carefully study the diagram below. It shows what your electrophoresis apparatus should look like when it is fully set-up and ready to run:

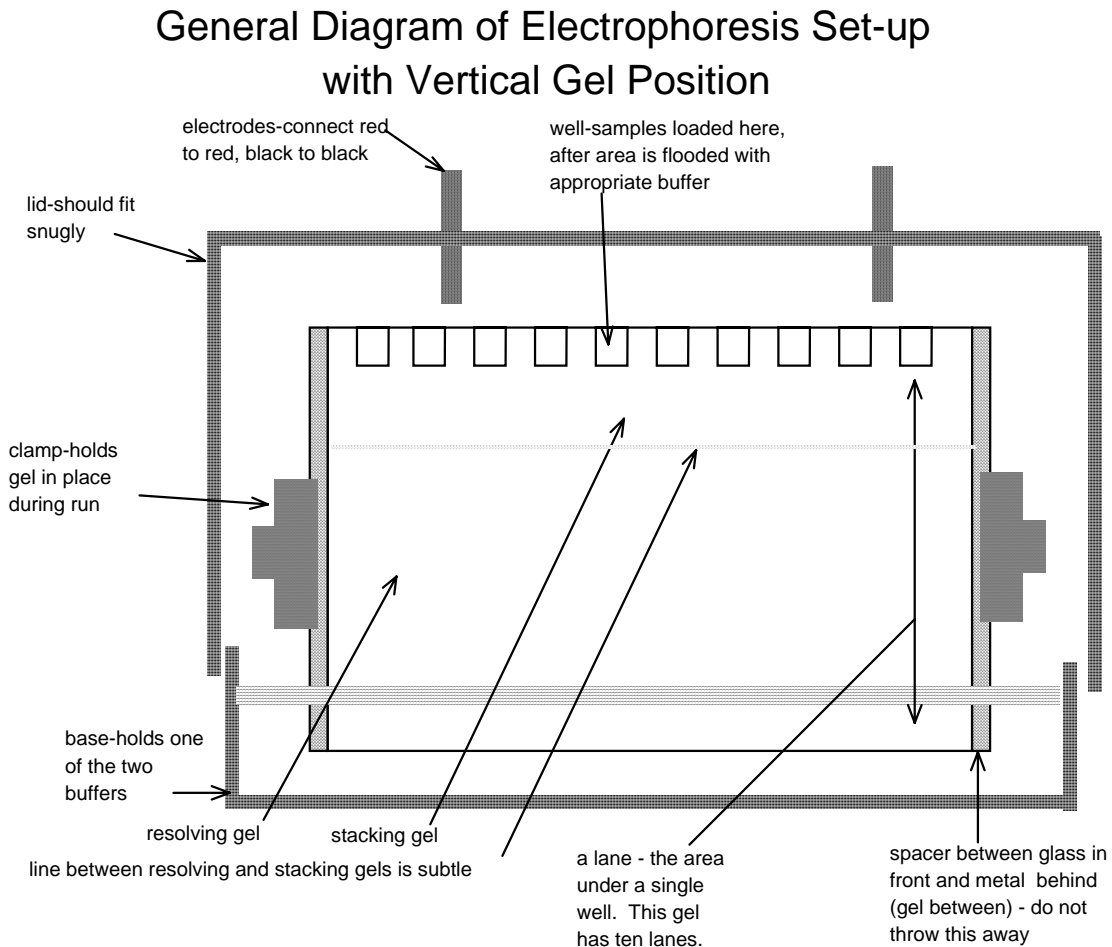


Diagram courtesy of Steve Bostic, ACC faculty

- With gloved hands, carefully unwrap the cellophane from your electrophoresis gel. Notice that the actual gel is sandwiched between a white metal plate and a glass plate. Two gray plastic spacers are located on either side of the gel, and a white plastic comb is sticking out from the top of the gel. This comb was used to form the wells (indentations) in the top of the gel.
- Handle the gel sandwich gently to avoid distorting or breaking the gel. Carefully remove the white plastic comb from the gel and drain any excess water out of the wells. Wash and save the gel comb - do NOT throw it away. Now clamp the entire gel sandwich onto the electrophoresis apparatus, with the metal plate pushed firmly against the rubber gasket.
- Place a clear plastic template on the glass plate and align the black lines with the wells in the gel. This will allow you to locate the wells once they have been filled with cathode buffer.
- Use a Pasteur pipette to fill the wells with "cathode buffer." Force the liquid into the wells with some velocity in order to sweep any non-polymerized gel components out of the wells. No harm is done if some Cathode Buffer splashes or overflows into the "top" reservoir (the narrow space behind the gel support chamber) since this reservoir will be filled with the same solution.
- Pour cathode buffer into the "top" reservoir until the liquid level is 3 – 4 mm below the top of the glass plate. This is necessary in order to make electrical contact between the electrode and the gel.
- Check to make sure that the cathode buffer is not leaking down from the upper buffer chamber. If it is leaking, add vacuum grease to the rubber seal and adjust your clamps to stop the leak before continuing with the experiment.

II. Prepare your samples and load them into the wells of your gel

- Last week you saved 8 Eppendorf tubes containing samples of your milk fractions with 2X sample treatment buffer added. These tubes have been placed in an ice bucket along with an additional Eppendorf tube containing a mixture of Molecular Weight Standards ("MW").
- If you need to dilute any of your samples before loading them into your gel, make your dilutions now. Make your dilutions according to the table below, which you completed as part of the Prelab:

Dilution of Milk Fractions to Make 40 μL of Diluted Sample with a Protein Concentration of 1.25 $\mu\text{g}/\mu\text{L}$		
Milk fraction	Amount of milk fraction with 2X sample treatment buffer needed (μL)	Amount of 1X sample treatment buffer needed (μL)
Skim milk		
Pellet		
Whey		
Column fraction _____		
Column fraction _____		
Column fraction _____		
Column fraction _____		
Column fraction _____		

3. Insert all **9** Eppendorf tubes (milk fractions plus MW standards) into a holder and place them in the 60° C water bath. Make sure you use the diluted samples for those fractions that required dilution, and the undiluted samples for those fractions that did not require dilution. Heat the samples for 5 minutes, removing them briefly at 1 minute intervals to mix by tapping the sides of the tubes. After 5 minutes, dry the Eppendorf tubes and place them in the microcentrifuge in balanced positions. Centrifuge for 5 sec to force the solutions to the bottoms.
4. Return the Eppendorf tubes to the ice bucket and leave them undisturbed to allow any aggregated proteins and insoluble particulate matter to settle.
5. Your gel contains 10 wells. The wells are identified as #1 through #10, starting with #1 on your left as you face the front of the gel. Following the instructions below, load 16 μ L of sample into each well.

Important: Load your samples carefully, to make sure that each sample stays in its proper well.

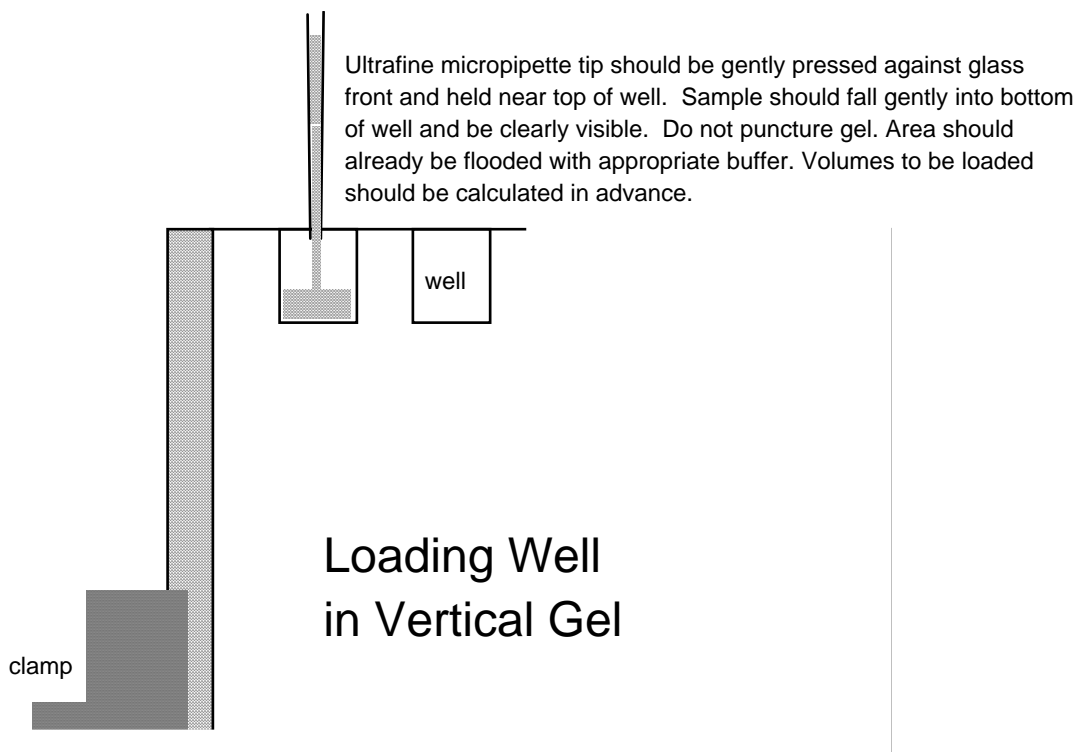


Diagram courtesy of Steve Bostic, ACC biology faculty

To load your first sample, adjust an automatic pipettor to deliver 16 μ L and attach an ultra-thin disposable capillary tip. Withdraw the correct amount of your milk sample from your Eppendorf tube labeled “Milk” and insert the tip of the pipettor into the top of well #1. Make sure that the pipettor tip is between the glass and metal plates of the gel sandwich. Keep the tip at least 4mm above the bottom of the well. Take care not to puncture the sides or the bottom of the well with your pipettor tip. This takes a steady hand – it may help to support the micropipettor with your other hand and to support your elbows on the lab bench top.

Very slowly and gently expel the solution from the pipettor tip into the well while holding the pipettor steady. The blue solution should fall to the bottom of the well, gradually filling it. **Do not press the pipettor to the second stop – it is important to avoid blowing air bubbles into the well. Do not release your thumb from the pipettor until you have completely withdrawn the tip from the well.**

If the sample overflows into an adjacent well, you may be trying to load too much sample (do not exceed 16 μ L). Alternatively, you may be expelling the sample with too much force, or you may be inserting the pipettor tip too far into the well so there is not enough room for your sample as it fills the well.

6. Place a new tip on the automatic pipettor, and then follow the procedure just described to load 16 μL of “MW standards” into wells 2 and 9.
7. Use the same procedure to load the other milk samples into the remaining wells on the gel. Be sure to use a clean tip for each protein sample, and keep a record of which sample was loaded into each well.

III. Run your gel

1. Remove the template from the front of the glass plate, and then pour anode buffer into the bottom reservoir. Wipe up any spills with paper towels so that your work area is dry. Place the protective plastic cover over the electrophoresis apparatus. Make sure the cover fits snugly.

Electric shock hazard! Be sure to follow instructions exactly!

2. **First make sure that the power supply is NOT connected to your electrophoresis apparatus.**
3. Plug in the power supply, turn it on, and adjust the voltage to 150V. It may be hard to get it to stay at exactly 150V, but get as close to that setting as possible. Once the voltage has been adjusted, **turn off the power supply and unplug it.**
4. As demonstrated by your instructor, connect the electrophoresis apparatus to the power supply and then plug in the power supply. Have your instructor check your set-up and connections. When given the OK by your instructor, turn on the power supply. Record the time when the power was turned on.
5. Running time is generally between 50 minutes and one hour. As current flows through the gel, proteins that are negatively charged will be pulled towards the bottom of the gel. You will not be able to see the proteins since they are colorless, but the blue tracking dye should remain visible. A few minutes after the power is applied, the blue bands should concentrate as a thin line below each well at the interface between the stacking gel and the resolving gel. The blue bands should then move slowly down through the resolving gel gradually becoming thicker as they move downward.
6. When the blue dye is within 2-3 millimeters of the bottom of the gel, turn off the power supply and unplug it. Finally, disconnect the power supply from the electrophoresis apparatus.

IV. Remove your gel and stain it with Coomassie Blue Solution

1. With the apparatus disconnected from the power supply, remove the cover from the electrophoresis apparatus. With gloved hands, carry the apparatus to the sink, secure the central section with your thumbs, and invert the apparatus to discard the buffer from the two reservoirs.
2. Return the electrophoresis apparatus to your work area. Place a piece of plastic wrap on your table. Remove the clamps holding the “gel sandwich” in place, and lay the gel sandwich on the plastic wrap with the glass plate on top.
3. Slide the two gray Teflon® spacers out from between the plates of the gel sandwich.

Wash and save the Teflon® spacers - do NOT throw them away!

4. Insert a spatula a short distance into the space where the Teflon spacers were and gently pry the top (glass) plate away from the gel. After you remove the glass plate, the gel should remain on the bottom (metal) plate.
5. Use the spatula to cut and discard a small triangular piece (approximately 5 mm on a side) from the lower right corner of the gel. Later, this will allow you to identify the lower right corner of the gel.

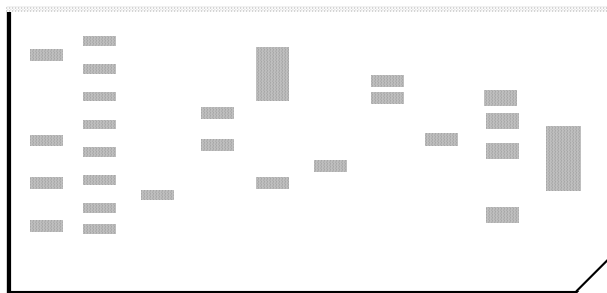
6. You should see a faint seam between the stacking gel (on top) and the resolving gel (on the bottom). Cut along this seam with a spatula and scrap away the stacking gel while leaving the resolving gel on the metal plate.
7. The resolving gel must be transferred to a Petri dish containing Coomassie Blue staining solution in order to stain the protein bands on the gel. The gel is very thin and fragile, so take care not to tear it. Use a dH₂O squeeze bottle to wet the gel. Hold the metal plate with the resolving gel upside down, just above the Petri dish containing Coomassie Blue solution. Using a spatula, gently peel the gel from the plate starting with the edge furthest away from the dye. Gently pull the corner of the gel away from the metal plate until it begins to peel away from the plate. Once the gel starts peeling from the plate it should fall into the Coomassie Blue solution without any help from you. When the gel is in the staining solution, cover the Petri dish and use a piece of tape to label it with your group name.
8. Place the dish on a rotary agitator and allow it to agitate slowly for 30 min.

While the gel is staining, wash the Teflon® spacers, template, and metal and glass gel plates, being careful not to scratch them. Give them a final rinse with dH₂O, and leave them on absorbent toweling to dry.

9. Rinse out the electrophoresis apparatus with tap water and then with dH₂O. Turn it upside down on a paper towel at your work station to drain.



Gel as it is likely to appear near end of run-- Fuzzy line near bottom is tracking dye; proteins are not yet visible.



Gel as it is likely to appear after staining and de-staining; Note that resolving gel has been removed, a notch has been cut below lane ten, and numerous bands (each a single protein) have appeared. Lanes 5 and 10 contain smears. Lane 1 contains 4 proteins; lane 2 contains 8 proteins and gives the general appearance of "markers"--molecular weight standards.

Diagrams courtesy of Steve Bostic, ACC biology faculty

V. Remove background stain from the gel

1. The Coomassie Blue stain will be reused, so after the gel has been in the stain for 30 minutes, pour the stain through the filter-lined funnel into the discard bottle, while carefully holding the gel in place with your gloved hand. Pour out as much of the stain as possible.
2. Deliver several milliliters of dH₂O from a squeeze bottle to the Petri dish containing the gel, swirl briefly to rinse the gel, and again holding the gel in the dish with your gloved hand, discard the rinse water into the sink.
3. Pour fresh "Destaining Solution" into the Petri dish containing the gel until it is half full. This solution is 50% Methanol + 40% dH₂O + 10% Acetic acid. Return the Petri dish to the agitator and record the time when destaining was begun.
4. After 15 minutes, pour the destaining solution from the Petri dish into the bottle labeled "Used Destaining Solution". Once again, pour fresh "Destaining Solution" into the Petri dish until it is half full.
5. After 15 minutes, pour the destaining solution from the Petri dish into the bottle labeled "Used Destaining Solution." Ask your instructor to examine the gel and determine if it requires additional destaining. The proteins within the gel should appear as blue horizontal bands in otherwise colorless lanes. If it is sufficiently destained, fill the Petri dish containing the gel about half-full with dH₂O.
6. Tape the Petri dish closed, and make sure it is labeled with your instructor's name, your lab day and time, and your group name. Give the dish to your instructor, who will store it until your next lab period.

Clean up

Be careful not to lose any parts of the gel apparatus, including the plates, the template, the spacers, and the gel comb. Wash them in soapy water, rinse with tap water and dH₂O, and leave on absorbent toweling to dry.

Dispose of your excess solutions as directed by instructor.

Remove label tape and any marks made with a marking pen from all glassware. Wash and rinse all glassware, give it a final rinse with dH₂O, and leave it inverted at your work area in order to drain.

All disposable glassware goes into the special glass disposal receptacle.

All instruments should be turned off and unplugged.

Wipe off your workspace with a damp paper towel.

Make sure everything that you have used is clean, put away, or discarded. Leave your work area in the same order that you found it in.

Ask your instructor to check your work area before you leave.

Postlab

1. Describe any difficulties that you had with the sample dilution, the gel loading, and the gel staining techniques.
2. What is the purpose of SDS-PAGE in this lab exercise?
3. When your gel is placed in the electrophoresis apparatus and the power is turned on, the wire in the upper reservoir serves as a negative electrode (cathode), and the wire in the bottom reservoir serves as a positive electrode (anode). Some of the major components present in the gel, including those drawn into the gel from the wells, are listed below. For each component listed, indicate whether it was drawn toward the anode, the cathode, or neither:

Hydrogen ion (H^+)

Tris⁺ (the buffer)

Hydroxyl ion (OH^-)

Water

Chloride ion (Cl^-)

Glycerol (polar)

Bromophenol blue (the tracking dye)

Sodium dodecyl sulfate (SDS)

Protein with many SDS molecules bound to it

4. Explain the functions of the following components of the 2X sample treatment buffer that was added to your milk fractions before loading them onto the electrophoresis gel:

SDS

Mercaptoethanol

blue dye

glycerol

5. Describe and **explain** how each of the following changes would affect the migration distance of α -lactalbumin during SDS-PAGE:
 - a. Increasing the ionic strength of the gel buffers.
 - b. Decreasing the electrophoresis run time.
 - c. Increasing the concentration of bis-acrylamide in the gels.
 - d. Increasing the pore size of the gels.
6. Compare gel exclusion chromatography to SDS-PAGE with respect to the following:
 - a. What is the basis for protein separation?
 - b. Which one maintains protein activity?
 - c. Which one destroys protein activity? Explain when, why, and how.