

**Lab #2:**  
**Comparison of Protein Content from Real and Imitation Crab Meat**  
**Using SDS Polyacrylamide Gel for Protein Electrophoresis**  
**(SDS-PAGE)**

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## **Lab #2: Comparison of Protein Content from Real and Imitation Crab Meat Using SDS Polyacrylamide Gel for Protein Electrophoresis**

### **Introduction**

The purpose of this experiment is to compare the protein content of real and imitation crab meat using a SDS polyacrylamide gel for protein electrophoresis. This experiment includes isolating and denaturing the protein samples, running the samples on a SDS-PAGE, staining and decolorizing the gel, and photographing it. This lab will be performed by the Lead Group before taking the class through the lab. The portions of the experiment that will be performed by the class during lab include:

- Isolating Total Protein from both Crab Meats
- Denaturing the Protein with Dithiothreitol and Heat
- Loading Sample on the Gel
- Observing the Protein Electrophoresis
- Staining and Destaining the Gel
- Analyzing the Gel

On the day of the lab, the Lead Group will have prepared all buffers and solutions for the class and set up the gel (a commercially made Bio-Rad® gel) and electrophoresis tank. The Lead Group will also be responsible for running, staining, and decolorizing the gel. A drawing and/or photograph of the gel will be taken and distributed to the class, or posted on the Internet. It is suggested that the Lead Group prepare a handout to give to the class that contains instructions specific to the sections of the lab they will perform.

### **Materials and Methods for Lead Group**

This experiment consists of the following sections:

Before Beginning	Loading the Gel
Preparing the Buffers and Solutions	Running the Gel
Sample Preparation	Staining the Gel
1. Isolation	Destaining the Gel
2. Denaturation	
3. Addition of Loading Dye	Analyzing the Gel
Preparing the Gel and Electrophoresis Tank	

#### **I. Before Beginning**

1. Preheat water bath to 70 °C.
2. Obtain 24 - 1.5 mL centrifuge tubes.
3. Prepare all buffers and solutions.

## Materials (continued)

Deionized water	Imitation crab meat	1000 mL flask
Glycerol	Bromophenol blue	Coomassie Destainer
Coomassie blue	3 – 50 mL beakers	2 - 100 mL volumetric flasks
Real crab meat	Dithiothreitol	Scale
10X Tris/Glycine/SDS buffer	Styrofoam floats	Staining trays
Polyacrylamide gel	Forceps	Pipettes and tips
Electrophoresis chamber	Spatula	Power supply
Gloves	Water bath	Mortar and pestle
Molecular weight standard	1.5 mL Centrifuge tubes	Centrifuge
Imaging system	Loading dye	

## II. Preparing the Buffers and Solutions

### **\*1X Laemmli Running Buffer (Tris/Glycine/SDS)**

Dilute stock 10X buffer: 100mL of 10X Tris/Glycine/SDS buffer with 900 mL deionized water in a 1000 mL flask.

(Note: 1 mL of this solution will be added to both ground-up crab meats originally, 7.30 mL will be used in Sample Preparation buffer, and 300-500 mL to fill the tank before running the gel electrophoresis.)

Do not adjust pH with acid or base. Store at 4 °C. If precipitation occurs, warm to room temperature before use.

### **\*\*0.5% Bromophenol Blue**

Dissolve 0.5g of bromophenol blue in 100 mL of deionized water in a 100 mL volumetric flask in order to obtain a 0.5% bromophenol blue solution.

(Note: Each lab group will need 200 µL from this solution.)

### **\*\*\*5 mM Dithiothreitol**

Dissolve 0.771g dithiothreitol in enough deionized water to bring total volume up to 100 mL in a 100 mL volumetric flask.

(Note: Each lab group will need 500 µL from this solution.)

### **Sample Preparation Buffer**

7.30 mL	1X Laemmli Running Buffer (Tris/Glycine/SDS)*
2.50 mL	glycerol (use 10 mL graduated cylinder to measure)
0.20 mL	0.5% bromophenol blue**
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10.0 mL	total volume

Mix above reagents and store at room temperature. On the day of the experiment, add 500 µL of 5 mM dithiothreitol\*\*\* to the 10.0 mL Sample Preparation Buffer.

### **Staining Solution:**

Commercially-made Coomassie Blue stock solution

### **Destaining Solution:**

Commercially-made Coomassie Destaining stock solution

## III. Sample Preparation

### **1. Isolation**

1. Weigh 0.75 g of the real Snow Crab claw meat.
2. Grind meat with mortar and pestle until it has a uniform consistency.
3. Transfer the ground meat into a 1.5 mL centrifuge tube (label).
4. Add 1000  $\mu$ l 1X Tris/Glycine/SDS\* buffer to tube.
5. Rinse mortar and pestle with 95-100% ethanol to avoid cross contamination of crab meats.
6. Repeat steps 1-4 for the imitation crab meat.
7. Centrifuge tubes for 5 minutes at 6,000 rpm.
8. Remove 250  $\mu$ L supernatant from each tube and transfer to a new tube (label for each).

### **2. Denaturation**

1. Add 750  $\mu$ l Sample Preparation Buffer (with dithiothreitol) to each tube from Step 8.
2. Place tubes into styrofoam float and place into a 70 °C water bath for 5 minutes to denature proteins.

### **3. Addition of Loading Dye**

1. Pipette 20  $\mu$ L from each sample tube from previous denaturation step into new 1.5 mL centrifuge tubes (label for each).
2. Add 5  $\mu$ L of loading dye into each tube (total volume = 25  $\mu$ L).

## IV. Preparing the Gel and Electrophoresis Tank

**WARNING: The acrylamide gel is a neurotoxin! It affects the nervous system and may cause death!! Gloves must be worn before proceeding with the following steps!!!**

1. Obtain the polyacrylamide gel (Bio-Rad® gel) from the lab instructors, remove the combs from the top and the clear scotch tape from the bottom (use razor to cut along the black line before removing), and place the gel into the vertical electrophoresis chamber so that the wells face the **INNER** chamber (or away from you). Gels can be loaded into either side of the electrode assembly.
2. Fill the tank with 1X Laemmli Running Buffer (300-500 mL). Add enough buffer to cover the top of the gel (center), but do not overfill the tank.

## V. Loading the Gel

1. Load well #1 (far left) with 25  $\mu$ L Bio-Rad® Precision Plus molecular weight standard.
2. Load all 25  $\mu$ l from the sample tubes with the added loading dye in the following order:
  - 1: MW Standard Ladder
  - 2: SKIP
  - 3 – 10: Real/Imitation protein from each 4 groups.

## VI. Running the Gel

1. Place the cover on the electrophoresis chamber and plug the leads into the power supply.
2. Verify that the power supply is plugged in and that the voltage is turned all the way down.
3. Turn on the power and dial the voltage up to 200 V or HIGH. There should be tiny bubbles rising from the bottom of the inner chamber. The sample can be seen to move slowly down the gel.
4. Run the electrophoresis for about 1 hour or until the tracking dye front reaches the end of the gel.
5. Turn off power supply once the tracking dye has reached the end of the gel.
6. Once power is off, disconnect the leads from the power supply and remove the cover of the gel chamber.
7. Return used buffer to buffer container.

## VII. Staining the Gel

1. Fill the staining tray with Coomassie Blue staining solution.
2. Using gloves, carefully remove gel plates from the electrophoresis chamber.
3. The gel will adhere to one of the plates. Carefully lift the other plate off of the plate containing the gel.
4. Invert the plate with the gel attached into the staining solution. The gel should float off of the glass plate into the staining solution.
5. Cover the tray with plastic wrap and let the gel stain overnight.

## VIII. Destaining the Gel

1. After staining, pour out Coomassie Blue staining solution and cover gel with the Destaining Solution.
2. Destaining solution must be changed at least twice prior to storing the gel overnight in the destaining solution.

## IX. Analyzing the Gel

1. Place finished gel onto light box and examine banding patterns.
2. Compare bands for the real and imitation crab meat to molecular standards to determine protein content.
3. List conclusions regarding banding patterns of imitation versus real crab meat.