SDS PAGE and Western Blot Protocol

Laboratory - Day 1

- Bring in fish samples.
- Analyze relatedness among samples using the phylogenetic charts.
- Complete Laboratory Day 1 protocol
  - Laemmli sample buffer
    - Solubilize the proteins in the fish muscle samples
    - Tris buffer, SDS, bromophenol blue, and glycerol.
  - Molecular weight marker
  - Actin and myosin standard
Lab - Day 1: Extract and Denature Fish Proteins

2. Label one 1.5 ml flip-top microtube for each of five fish samples. Also label one screwcap microtube for each fish sample.

3. Add 250 μl of Bio-Rad Læmmli sample buffer to each labeled flip-top microtube.

4. Cut a piece of each fish muscle about 0.25 x 0.25 x 0.25 cm³ and transfer each piece into a labeled flip-top micro test tube. Close the lids.

5. Flick the microtubes 15 times to agitate the tissue in the sample buffer.

6. Incubate for 5 minutes at room temperature.

7. Carefully transfer the buffer by pouring from each flip-top microtube into a labeled screwcap microtube. Do not transfer the fish.

8. Obtain the Kalleidoscope prestained standards (KGS) and the actin and myosin standard from your teacher.

9. Heat the fish samples and the actin and myosin standard (AM) in screwcap microtubes for 5 minutes at 95°C.

Lab Day 2: Gel loading, running and staining

• Defrost the protein extracts.
• Prepare the electrophoresis chamber
• Prepare the pre-cast gel and lock it into the electrophoresis chamber.
• Load and run the gel.
  – 1x TGS buffer → Tris-glycine-SDS
• Stain the gel
  – Coomassie stain
**Mini-PROTEAN 3 Electrophoresis Module Assembly**

1. Prepare a Ready Gel cassette by cutting along the black line on the bottom of the cassette with a razor blade and pulling off the plastic strip, as indicated on gel cassette.

2. Remove the comb from the Ready Gel cassette.

3. Place Ready Gel cassette into the electrode assembly with the short plate facing inward. Place a buffer dam or another Ready Gel cassette on the opposite side of the electrode assembly, with notch on buffer dam facing inward.

4. Slide gel cassette, buffer dam, and electrode assembly into the clamping frame.

5. Press down the electrode assembly while closing the two cam levers of the clamping frame.

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**Lab Day 2: Prepare Chamber**

6. Lower the inner chamber into the mini tank.

7. Completely fill the inner chamber with 1x TGS electrophoresis buffer, making sure the buffer covers the short plate (~150 ml).

8. Fill mini tank with approximately 200 ml of 1x TGS electrophoresis buffer.

9. Place sample loading guide on top of the electrode assembly.

10. Load samples and run gel at 200 V for 30 minutes.
Lab Day 2: Gel loading, running and staining

**Change volume to 5ul for all samples

10. Load your gel:

<table>
<thead>
<tr>
<th>Lane</th>
<th>Volume</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 &amp; 2</td>
<td>empty</td>
<td>empty</td>
</tr>
<tr>
<td>3</td>
<td>10 µl</td>
<td>Kaleidoscope standards (KS)</td>
</tr>
<tr>
<td>4</td>
<td>10 µl</td>
<td>fish sample 1</td>
</tr>
<tr>
<td>5</td>
<td>10 µl</td>
<td>fish sample 2</td>
</tr>
<tr>
<td>6</td>
<td>10 µl</td>
<td>fish sample 3</td>
</tr>
<tr>
<td>7</td>
<td>10 µl</td>
<td>fish sample 4</td>
</tr>
<tr>
<td>8</td>
<td>10 µl</td>
<td>fish sample 5</td>
</tr>
<tr>
<td>9</td>
<td>10 µl</td>
<td>Actin and myosin standard (AM)</td>
</tr>
<tr>
<td>10</td>
<td>empty</td>
<td>empty</td>
</tr>
</tbody>
</table>

11. Electrophorese for 30 minutes at 200 V in 1x TGS electrophoresis buffer.
Lab Day 2: Stain the gel

1. When gels are finished running, turn off the power supply and disconnect the leads. Remove the lid and lift out the electrode assembly and clamping frame.

   **DO NOT DISCARD THE RUNNING BUFFER!**

2. Pour out the running buffer from the electrode assembly. Open the cams and remove the gel cassettes.

3. To keep the gel free of contamination from your fingertips, wear gloves to handle the gels from this point on. Lay a gel cassette flat on the bench with the short plate facing up. Cut the tape along the sides of the gel cassette. Carefully pry apart the gel plates, using a spatula or your fingertips. The gel will usually adhere to one of the plates. Transfer the plate with the gel adhering to it to a tray containing Bio-Safe Coomassie Blue stain, allowing the liquid to detach the gel from the plate. The gel may also be lifted directly (and gently!) from the plate and placed into the stain.

4. Allow the gels to stain for 1 hour, with shaking if available.

5. Your teacher will discard the stain and replace it with a large volume of water to destain the gel overnight.

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LAB DAY 3: Western Blotting

1. Using a ruler, chop the top and bottom off the gel.

2. Equilibrate the gel in blotting buffer for 15 minutes on a rocking platform.


4. Mark the white nitrocellulose membrane with penciled (or black ball point pen) initials and prewet in blotting buffer along with the blotting paper.
LAB DAY 3: Western Blotting

5. Make the blotting sandwich:
   a. Add 1 cm depth of blotting buffer to container and insert plastic cassette with black side down.
   b. Lay a wet fiber pad on the black side of the cassette.
   c. Lay one wet blotting paper on the fiber pad and roll out air bubbles.
   d. Lay gel squarely on blotting paper and roll out air bubbles.
   e. Lay wet nitrocellulose membrane on the gel and roll out air bubbles.
   f. Lay one wet blotting paper on the membrane and roll out air bubbles.
   g. Lay a wet fiber pad on top of the blotting paper.
   h. Close the cassette and clamp together with the white clip.

LAB DAY 3: Western Blotting
LAB DAY 3: Western Blotting

6. Set up the Mini Trans-Blot module with the black side of the cassette next to the black side of the Mini Trans-Blot module. Add a frozen Bio-Ice module and fill with blotting buffer up to the white clip.

7. Place lid on tank, matching the power cords red-to-red and black-to-black, then blot at 20 V for 2.5 hours.

8. At this point the blots can be stored in the tanks submerged in blotting buffer at room temperature overnight or the sandwiches dismantled and the blots placed in blocker overnight at 4°C.
LAB DAY 4: Immunodetection for Myosin Light Chains

Lesson 4 Quick Guide

1. If not blocked overnight, immerse membrane in 25 ml blocking solution for 15 minutes to 2 hours at room temperature on a rocking platform.

2. Discard blocking solution and incubate membrane with 10 ml of primary antibody for 10–20 minutes on rocking platform set to a faster setting to ensure constant coverage of the membrane.

3. Quickly rinse the membrane in 50 ml of wash buffer then discard the wash.

4. Add 50 ml of wash buffer to membrane for 3 minutes on rocking platform at a medium speed setting.

5. Discard the wash and incubate membrane with 10 ml of secondary antibody for 5–15 minutes on rocking platform set to a fast setting.
LAB DAY 4: Immunodetection for Myosin Light Chains

6. Quickly rinse the membrane in 50 ml of wash buffer and discard the wash.
7. Add 50 ml of wash buffer and wash membrane for 3 minutes on rocking platform on a medium speed setting.
8. Discard the wash and add 10 ml of HRP color detection reagent.
9. Incubate 10–30 minutes, either with manual shaking or on a rocking platform, and watch the color development.
10. Rinse the membrane twice with distilled water and blot dry with paper towel.
11. Air dry for 30 minutes to 1 hour and then cover in plastic wrap or tape in lab book.

Lab Day 5: Analysis

- Take a picture of your gel.
- Analyze your results.