

Ch 8 Revamp – Week 2

LEARNING GOALS:

- To analyze the synthesized proteins by SDS-PAGE.
- To observe the synthesized proteins by autoradiography.

IN YOUR PRELAB NOTEBOOK PAGES, INCLUDE:

- Everything normally required for the class, as well as
- A way to keep track of the way you loaded your gel

HAZARDS

Gel solutions: CAUTION! Acrylamide is neurotoxic. Always wear gloves when working with acrylamide solutions and avoid any contact. If you splash any on your skin, wash it off with large quantities of water. All glassware that has been in contact with acrylamide should be thoroughly rinsed. Once polymerized, however, acrylamide is not toxic.

PROCEDURES

Second Week (Part C)

Remember, last week you worked in big groups, then split into smaller groups at the end to make your SDS-PAGE samples.

You will thaw your samples from the previous lab section, which should have already been mixed with 2x SDS-PAGE loading dye. Keeping the tubes covered to avoid bleaching your fluorophore, let thaw at room temperature and spin them down to ensure all the sample is on the bottom of the tube.

While thawing, obtain a 10% polyacrylamide gel poured by either the TFs or the previous section from the fridge. Set up the gel running apparatus as described in Ch 5., remembering to share the gel box with the other half of your big group. Load 5 μL of the provided ladder (likely <https://www.thermofisher.com/order/catalog/product/26619>).

Load 20 μL of each of your four reactions from last week – remember, we are **not** boiling these samples before loading them. The Fluortect Green_{Lys} is not stable above $\sim 70^\circ\text{C}$. You will have to slowly pipet each of these samples into each well to make sure the sample does not spill into the neighboring lanes. You might not be able to fit 100% of the 20 μL in each lane. This is acceptable as we are not using this gel in a quantitative way.

Run your gels at 80 V through the stacking buffer, then 180 V until the dye front lines up with the green and tan bottom of the gasket. While the gel is running, cover the gel box with a cardboard box to avoid bleaching the fluorophore. Alternatively, wrap the gel box in aluminum foil. Only lift the box to check the progress of the dye front through the gel.

While your gel is running, cast a 10% polyacrylamide gel for the next group of students.

Table I: Resolving Gel Solution for a 10% polyacrylamide gel

Stock Solution	Volume per gel (mL)
1.0 M Tris-HCl, pH 8.8	1.9
40 % (w/v) acrylamide, 1.5% (w/v) bisacrylamide	1.25
10 % (w/v) SDS	0.05
Water	1.8
Ammonium persulfate, 100 mg/mL (10% w/v)	0.05
TEMED (tetramethylenediamine)	0.005
TOTAL	5.0

Mix all ingredients except TEMED and ammonium persulfate in a 50-mL conical tube. Add ammonium persulfate and TEMED as described in the recipe above, mix by swirling, and pour the solution between glass plates to within 2 cm of the glass assembly's top (about ½ a cm below the green line of the apparatus that holds the glass plates together). Overlay with isopropyl alcohol. Allow 15–30 min to polymerize. Again, leave the remainder in the conical tube and allow that to polymerize. This both detoxifies the remainder and is used to determine when your gel has polymerized.

Table II: Stacking Gel Solution

Stock Solution	Volume per gel (mL)
1.0 M Tris-HCl, pH 6.8	0.63
40 % (w/v) acrylamide, 1.5% (w/v) bisacrylamide	0.56
10 % (w/v) SDS	0.05
0.5 M EDTA, pH 8.0	0.01
Water	3.695
Ammonium persulfate, 100 mg/mL (10% w/v)	0.05
TEMED (tetramethylenediamine)	0.005
TOTAL	5.0

Mix all ingredients except TEMED and ammonium persulfate in a 50-mL conical tube. After the running gel has polymerized, drain off the isopropyl alcohol well, and insert a clean, dry comb halfway down. There should be about 1 cm between the ends of the “fingers” that will form the wells and the top of the running gel. Be sure to leave some space on one side to pour the gel solution. Add the TEMED and ammonium persulfate as described in the recipe above, then mix by swirling. Slowly apply the solution with a pipette down one side until the spaces between the wells are full. Be certain that any bubbles are removed at the end of the “fingers.” The gel solution should be overflowing the edge of the plate. Let polymerize. Again, leave the remainder in the tube to polymerize. After polymerization is evident, pull out the comb and rinse the sample wells with water using a wash bottle.

Present the gel to your TF for inspection. If deemed satisfactory, wrap the gel gently in a damp paper towel and store in the correct location in the fridge.

Obtain a 15 cm petri dish. On both the lid **and** the underside of the dish, write your group's name/number and your lab section.

Once the dye front has reached the green and tan bottom of the gasket (about 1

cm from the bottom of the gel itself), stop the current and disassemble the gel running box. Gently take the glass plates off of the gel and deposit the gel into the petri dish. Gently, wash the gel for about 30 sec under the DI water faucet. When you dump the water out, hold down your gel with a few fingers so it doesn't go splat in the bottom of the sink. Leave a little water in the petri dish so that your gel doesn't dry out, parafilm around the edge of it to keep the water in, and hand to your TF for imaging.

Fluorescence Imaging (TFs only):

1. Reserve the instrument in the online calendar (for an hour) to start about 2 h after your lab section starts.
2. In a box, bring over the students' gels to the room. Don't forget your USB.
3. Turn the imager on, start up the software.
4. After cleaning the stage, add a little DI water to the upper left hand corner where the gels will be placed. Enough so that the gels aren't put onto a very dry stage, not enough so that they slide around.
5. Keeping track of which is which, arrange the gels in the upper left hand corner in the bounds of the blue markings, leaving a little extra room on the edges.
6. Cover the gels with the black side of the pad facing down.
7. In the software, scan the entire blue-edge marked region with the pre-set BB422 Cy2 fluorescent program. Save as a TIFF file.
8. Open the lid of the instrument, flip the pad over so the white side touches the gels now.
9. Switch to 'visible' in the software, and once again scan the entire blue-edge marked region. Save as TIFF.
10. Repeat if necessary.
11. Make sure to clean the instrument and the space before you leave.
12. Using ImageJ or similar, crop the gel images to send to students. Use ROI manager or a similar tool to ensure the photo and the fluorescent scan are the same size and location.
13. Remember, never save images as JPG/JPEG – use TIFF as your first option and PNG as the second.
14. Upload images to the Google Drive, sharing links (only to the image, not to the folder or to the drive itself) with the relevant students.

SET UP AND MATERIALS:

Per large group:

- 1 Power supply
- 1 Bio Rad Mini-PROTEAN 3 Gel electrophoresis apparatus. This includes the lower buffer reservoir, the lid with cables attached, the inner clamp assembly, and the inner core with electrodes.

Per pair/small group:

- 1 glass plate (7 x 9 cm, with 0.75 mm spacers attached) for casting gels
 - 1 glass plate (5 x 9 cm) for casting gels
 - 1 comb, 10-place (0.75 mm thick)
 - 2 150-mm plastic petri dish
 - 1 ◊Pipetmen P-20, P-200 & P-1000 with tips for each
 - 2 Gel loading tips for Pipetman (or Hamilton syringes (50 μ L) fitted with thin bore polyethylene tubing (PE50))
- Disposable gloves
Kimwipes

Glassware (per pair /small group, unless stated otherwise):

- 2 50 mL conical tubes
- 3 5 mL glass pipets, serological

Reagents (per pair /small group, unless stated otherwise):

- 2 mL 1.0 M Tris-HCl, pH 8.8, 121 g Tris-base in water, pH to 8.8 with conc. HCl, fill to 1 L.
- 1 mL 1.0 M Tris-HCl, pH 6.8, 121 g Tris-base in water, pH to 6.8 with conc. HCl, fill to 1 L.
- 3 mL 40% (w/v) acrylamide, 1.5% (w/v) bisacrylamide. Dissolve 120 g acrylamide plus 4.5 g bisacrylamide in water and bring to 300 mL with water. Keep in brown bottle, cold; stable for months.
- 0.1 mL 10% (w/v) SDS. 10 g SDS in 100 mL water.
- 0.01 mL 0.5 M EDTA, pH 8.0. Dissolve 186 g Na_2EDTA in water, adjust pH to 8.0, fill to 1.0 L with water (see Chapter 6). Store at room temperature; stable for years.
- 15 μ L TEMED (tetramethylenediamine)
- 125 μ L Ammonium persulfate 10% (w/v), or 100 mg/mL. Dissolve 1 g to 10 mL with water. Store at 4 °C. MAKE FRESH every day.
- 3 per lab Isopropanol, in squirt bottles
- 80 mL Electrophoresis Buffer (10X): Dissolve 90 g of Tris-base, 432 g of glycine, and 30 g of SDS in water to make 3 liter of solution, pH to 8.3 with HCl if necessary. Store at room temperature; stable for years.

5 μ L

PageRuler™ Plus Prestained Protein Ladder, 10 to 250 kDa from Thermofisher , or similar. Ideally not the Bio-Rad Kaleidoscope ladder, as that has fluorescent bands. ALIQUOT into capped tubes.

Distribution of number of groups per section:

Section	B0	B1	B2	B3	B4	B5	All
Large groups	NA	3	4	4	4	3	18
Pairs or trios	NA	6	8	8	8	6	36