

Lab 11 SDS-PAGE and Protein Profiles

See attached material for background.

Purpose:

To improve the students' ability to run a protein gel and interpret the results

Procedure:

1. Obtain 5 microcentrifuge tubes and label A-E with your initials. Log in your lab book which fish corresponds to which letter.
2. Add 250 μ L of Laemmli sample buffer to each labeled tube.
3. For each sample, obtain a piece of fish muscle (avoid skin, fat, and bones) approximately 0.25 cm x 0.25 cm x 0.25 cm in size, and transfer it to the appropriately labeled microcentrifuge tube.
4. Gently flick the microcentrifuge tube 15 times with your finger to agitate the tissue in the sample buffer.
5. Incubate the samples for 5 minutes at room temperature to extract and solubilize the proteins.
6. Label 5 new microcentrifuge tubes the same as you did in step 1.
7. Transfer the buffer containing the extracted proteins, but not the solid fish piece, to the corresponding new microcentrifuge tube. Note: It's not necessary to transfer all the liquid to the new tube, since only a small volume ($<20 \mu$ L) is actually needed for gel loading.
8. Throw away the tubes with fish muscle tissue.
9. Obtain an actin & myosin (AM) standard and a protein ladder (PL) and attach snap on caps to all six tubes. Heat your fish samples and the standards for 5 minutes at 95°C to denature the proteins in preparation for electrophoresis.
10. Cool tubes on ice for 5 minutes, remove clips, and microfuge tubes 30 seconds to pull down any condensation on the tubes. Store at room temperature.
11. Obtain an SDS gel from the instructor and remove from wrapper. Cut the seal and pull off the adhesive tape as directed by the instructor.
12. Remove the gel comb under a stream of water.
13. Assemble protein gel apparatus with the SDS gel.
14. Fill inner gel tank with SDS running buffer and watch for leaks. If the gel apparatus is leaking, call the instructor over for help. If there is no leak, add SDS running buffer to the outer tank until it is about 1 inch deep.
15. Attach the electrodes to the power supply and run at 200 V until the dye front runs off the bottom of the gel.
16. Dump out SDS running buffer into the collection beaker and remove the gel cassette.
17. Cut the adhesive on both sides of the gel and separate plates of the gel cassette. Note: The SDS gels are very delicate and you need to proceed carefully so as to not rip the gel.
18. Gently peel the gel off of the plate it sticks to and transfer to the staining container and add the stain solution until the gel is completely covered.
19. See instructor for complete instructions to stain the gel.

Results:

You should tape in a picture of your SDS PAGE gel. Label all lanes and standard values.

Conclusion:

Were you able to separate and visualize proteins in a sample by size? Why or why not?
Do you think any of the fish are more closely related? Why or why not?