

Bioc 463A: 2011-2012

Experiment 5.1: SDS-PAGE Electrophoresis of Proteins.

Reading: Chapter 6 in NB&B

Review the following YouTube videos about setting up the gel plates and pouring the resolving and stacking gels:

http://www.youtube.com/view_play_list?p=0DF3730B1D55C58B

Procedure for Experiment 5.1: SDS PAGE and next week's Western Blots

(This is a more complete version of the protocol given in your text.)

In this lab you will be provided with a set of known proteins, a ladder of standard proteins. Your objectives are:

- 1) To become familiar with the techniques of SDS PAGE.
- 2) To determine (estimate) the molecular mass of the *E. coli* **beta-galactosidase** you purified last Thursday and *E. coli* **alkaline phosphatase** (AP) by comparison with the standards. (We might also throw in a mystery protein at the last minute). We will also run a lane of the beta-galactosidase cell lysate from last week's experiment.

Preparing a SDS Polyacrylamide Gel

IMPORTANT: Unpolymerized acrylamide is a potent neurotoxin.

1. Assemble the gel pouring apparatus. (This will vary depending on the gel system that you have.)
2. In order to minimize leakage, the TA's will pipette enough liquidified agarose (0.5%) to make a semipermeable plug at the bottom of your gel plates.
3. Mix the following resolving gel components: H₂O, 4X resolving buffer, 30% acrylamide, and 10% APS; **using ½ the volumes** given in **Table 6-2** in NB&B (p. 184) for a **10% SDS-PAGE gel**.
4. When you are absolutely CERTAIN you are ready to pour the gel, add the correct volume of TEMED according to Table 5-2. **Once TEMED is added, polymerization begins!!!!**
5. Pour the mixture into the gel pouring apparatus using a Pasteur pipette, leaving enough room for a decent sized stacking gel (about 1 cm from the bottom of the wells of the comb).
6. Gently apply a layer of H₂O to the top of the mixture to level the resolving gel.
7. Allow to solidify.
8. Once it has solidified pour off the H₂O. Remove any additional H₂O with a Kimwipe or extra filter paper.
9. Mix the following stacking gel components; 30% acrylamide, H₂O, 4X stacking buffer, and 10% APS; using ½ the volumes given in Table 6-3 (NB&B, p. 185).
10. Add the TEMED.
11. Insert the comb into the gel pouring apparatus.
12. Allow the gel to solidify.

Expt. 5: SDS and Native PAGE

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Preparing the protein samples:

1. Add three volumes of sample to one volume of 4X sample loading buffer in a 0.65 mL eppitube. (Do not treat the pre-stained Mol. Wt. Standards by this procedure. Use as is).
2. Heat these samples in boiling water bath for 5 min.
3. Centrifuge eppitubes for 60 seconds in mini-centrifuges to get condensation drops back into sample.
4. Load the samples and standards into the wells of the gel.

Running the Gel

1. Remove the comb from the gel pouring apparatus and place the gel into the gel running apparatus.
2. Add Electrophoresis buffer to the gel running apparatus.
3. Run the gel between 100 and 150 volts until the blue tracking dye has run off the bottom of the gel. The lower the voltage the better the resolution.

Staining and Destaining a SDS Polyacrylamide Gel

1. Remove the gel from the gel running apparatus.
2. Carefully remove the gel from the glass plates.
3. Place gel into an adequate amount of Coomassie fixing and staining solution.
4. Allow to stain for the desired amount of time.
5. Place gel into the 50:10 destaining solution. (There are typically two destaining solutions used. The 50:10 solution, containing 50% methanol and 10% acetic acid, is used to initially destain the gel. The 50% methanol will shrink the gel due to dehydration, so a second 10% MeOH:10% acetic acid solution is used to rehydrate and swell the gels).
6. After destaining, determine the molecular weights of the three proteins by plotting Log MWT vs. Relative Mobility for the standards and three proteins (see. Fig. 6-7, p. 174).

Procedure for Experiment 5.2: Native Polyacrylamide Gel Electrophoresis.

In this experiment, we will run two enzymes on the native gels, β -galactosidase and alkaline phosphatase (AP), as well as a couple of lanes of the beta-galactosidase cell lysate from last week's experiment. Using colorimetric assays we will attempt to detect the bands for both enzymes using assays that are specific for each enzyme. One half of the class will assay for β -galactosidase first using the ONPG/Z-buffer assay and then assay for alkaline phosphatase using the reaction described in Fig. 6-2 (p. 187). The second half of the class will assay for AP first, then assay for β -galactosidase second. After observing the results, you will discuss in small groups what you have observed and offer a reasonable explanation for the results.

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Procedure:

1. Pour a **7.5%** native PAGE gel, which does not contain SDS. Based on the volumes given in Tables 6-2 and 6-3 (NB&B), calculate the volumes for the resolving and stacking buffers, then adjust the volume of water appropriately.
2. For native gels, the protein samples are not boiled prior to loading (that's why they are called native gels) and a native loading buffer is used prior to addition of samples to the gel. Typically, ladders are not used with native gels. Volumes for pouring the gels are exactly the same as in SDS-PAGE, except you will use Native Resolving and Stacking buffers.
3. After tracking dye has run off bottom of gel, **CAREFULLY** remove the gel from the plates and place in the Petri plate lid containing a little H₂O.
4. Stain the gel with the appropriate assay solution and wait for color to develop.
5. Rinse the gel with water and assay for the second gel.

What to know from this week's experiments:

1. The fundamental procedures for pouring either a SDS or native PAGE gel. Next week we will do a Western transfer that **ALWAYS** begins with a SDS-PAGE gel (10%).
2. The chemicals involved in polymerization of a PAGE gel: acrylamide, bis-acrylamide, APS, and TEMED.
3. The function of SDS and BME in the loading buffer.
4. The molecular weight of monomeric AP determined from the SDS-PAGE gel. We will determine the mwt of monomeric AP by mass spectrometry in a subsequent set of exercises.
5. At the end of the semester, you will purify AP from *E. coli*, employing SDS-PAGE to check for purity of the protein fractions from each stage of the purification procedure, so these techniques will be revisited.