SDS-PAGE

Introduction:

Polyacrylamide gels are formed by the polymerization of **acrylamide** monomers and N,N'-methylene-bis-acrylamide (**Bis**), a cross-linking agent. The polymerization is initiated by the ammonium persulfate (**APS**), free radicals server, and catalyzed by N,N,N₁,N₁,-tetramethylethylenediamine (**TEMED**).The gels are neutral, hydrophillic, three-dimensional networks of long hydrocarbons.

Electrophoresis is the separation of charged molecules in an applied electric field. Electrophoresis in acrylamide gels is frequently referred to as PAGE, being an abbreviation for **p**oly**a**crylamide **g**el **e**lectrophoresis. When the detergent **SDS** (sodium dodecyl sulfate) is used, the procedure is abbreviated as **SDS-PAGE**. Originally, the charge of a protein can be either + or－, depending on the pH of the solution. But these SDS anionic molecules mask the native charge of the protein and become negatively charged, resulting in a constant charge density (the ratio of charge to mass), so the migration of protein-SDS complexes can be assumed to be inversely related to the molecular size (i.e. **molecular weight)**.

The separation is determined by the relative size of the pores formed within the gel, however the pore size of a gel is determined by the total amount of acrylamide present % (w/v) (**T %**) and the amount of cross-linker (**C %**). **The higher the concentration, the smaller the pore size** and the better it will be able to separate smaller

molecules, so it is dependent on the molecular weight of the protein to be separated.

$$T\ \% = (a + b) / \text{total volume (ml)} \times 100\%$$

$$C\ \% = b / (a + b) \times 100\%$$

(a = weight of acrylamide in g, b = weight of bis in g)

For good separation of proteins, **discontinuous gel systems** is used, the **stacking gel (or upper gel)**, which has lower acryamide concentration and pH, but bigger pore size than the **separating gel (or running, lower gel).** The proteins are concentrated into narrow bands in the stacking gel, however they continue to migrate in the separating gel with different mobility.

Sample buffer contains SDS, which binds strongly to, and denaturing the protein; β-mercaptoethanol, which reduces disulphide bridges; glycerol, which gives the sample solution density thus allowing the sample to settle easily to the bottom when injected into the loading well; a tracking dye, usually bromophenol blue, that allows the electrophoresis to be monitored.

When all the samples (protein marker) are loaded, a current is passed through the gel and the bands subsequently visualized using an appropriate dye, usually **Coomassie Blue R-250**.

Reagents:

1. 30% acrylamide/bis mixture (29:1)
2. Running gel buffer (pH 8.8): 1.5M Tris-HCl
3. Staking gel buffer (pH 6.8): 0.5M Tris-HCl
4. 10% sodium dodecylsulfate(SDS)
5. 10% ammonium persulfate(APS)
6. N,N,N',N'-TEMED
7. Tank buffer(10X Tris/Glycine/SDS)
8. Protein Marker

Procedure:

A. Gel Casting

1. Assemble the gel casket, frame and stand according to the operation instruction. Add H_2O to test for leakage.
2. Follow table 7.1, prepare the **running** gel solution in a 20mL vial by combining all reagents, APS and TEMED are added last. Mix the solution by swirling the container gently. (Acrylamide is a neutrotoxin, gloves should be worn at all times.)
3. Carefully introduce the solution into gel sandwich to the height ~2/3 by pipetting along a spacer. Immediately overlay the gel with spray bottle of alcohol to keep surface flat.
4. Allow gel to polymerize ~15 mins. Pour off water covering the running gel. Drain the excess water with strips of filter paper.
5. Prepare the **stacking** gel solution as before and pipet it onto separating gel until solution reaches top of front plate. Carefully

insert comb into gel sandwich. Allow the stacking gel to polymerize about 10 mins.

Table 7.1 Solutions for preparing gel

Components \ Volume(mL)		10% Running gel	5% Stacking gel
		10mL	5mL
1	H_2O	4ml	3.4ml
2	30% Acrylamide/bis mixture (29:1)	3.3ml	0.83ml
3	Tris-HCl	分離膠緩衝液 (1.5M , pH8.8) 2.5ml	濃縮膠緩衝液 (0.5M , pH6.8) 0.7ml
4	10% SDS	0.1ml	0.05ml
5	10% APS	0.1ml	0.05ml
6	TEMED	0.01ml	0.01ml

B. Sample loading

1. After stacking gel has polymerized, remove comb carefully, making sure not to tear the well ears.
2. Place gel into the inner chamber and fill it with tank buffer until the buffer reaching over ~ 0.1cm of the shorter glass plates.
3. Put sample loading guide in the correct place.
4. Introduce sample solution into well using a micropipette. Layer protein solution on bottom of well and raise tip vertically. (Be careful to avoid introducing air bubbles as this may allow some of sample to be carried to adjacent well.)

5. Record the numbers that the sample is loaded.

C. Gel Electrophoresis

1. Add tank buffer into the outer chamber.
2. Attach the electrical wires to power supply. (Note: black to black and red to red). Run the gel at constant voltage of **180 V**.
3. Stop it when the tracking dye is ~1cm above the end of the glass plates.

D. Stain, and destain the gel

1. After electrophoresis, turn off the power and carefully remove the gel from between the glass plates.
2. Cut the gel on one side of oblique angle (to orientate the gel).
3. Place it in the beaker containing the Coomassie Blue stain for ~10 min. **(Make sure that the gel is fully submerged in the solution.)**
4. Remove the gel from the stain solution and place in water for ~5 min until protein bands are visualized.
5. Put your gel on a piece of glass and take a photo for it with a digital camera. Print this image.

Results：

1. Record your results (take a photo and print this image).
2. Label the molecular weight of each band of standard.

Discussions：