

TEACHER REFERENCE NOTES-FISH PROTEIN LAB

Separation of proteins and nucleic acids from complex mixtures using polyacrylamide gel electrophoresis has become a versatile, widely used analytical technique. Gel electrophoresis can be performed under denaturing or nondenaturing conditions, depending on the choice of gel forming reagents and conditions, buffer systems, and temperature. The most common techniques for protein separation for analytical purposes is denaturing, discontinuous electrophoresis (SDS-discontinuous electrophoresis)

In a typical application, the polyacrylamide gels are formed by polymerization of acrylamide and a cross-linking reagent (such as N,N'-Methylene-bis-acrylamide). The pore size of the polyacrylamide matrix is determined by the concentration of the acrylamide and the amount of crosslinker used, and permits separation by size. In general, the higher the acrylamide content, the smaller the proteins that will be retained in the gel. Polymerization is accomplished by using an initiator (such as ammonium persulfate) and a catalyst (such as TEMED: N,N,N',N'-Tetramethylethylenediamine).

The sample to be studied is treated with a detergent, sodium dodecylsulfate (SDS), so that all the protein molecules in the sample have a net negative charge. Next, loading dye (containing a high specific gravity substance which allows the protein sample to settle to the bottom of the well in the gel, and a tracking dye to permit visualization of the migrating components at the leading edge) is added. After separation, the gel is stained to allow viewing of the various bands.

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These notes are intended for those who choose to run this protein lab on their own without TOPS support. These solutions will be prepared in advance by the TOPS staff, and you will be supplied with sufficient gels, buffer, stain and destain solutions for your classes. PLEASE BE AWARE THAT SOME OF THESE CHEMICALS ARE HIGHLY TOXIC. IF YOU CHOOSE TO RUN THIS LAB ON YOUR OWN, **BE SURE** TO OBSERVE ALL NEEDED PRECAUTIONS. THE PREPARATION FOR THIS LAB SHOULD **NOT** BE DONE BY STUDENTS.

A. PREPARATION OF STOCK SOLUTIONS FOR ACRYLAMIDE GELS

1. Monomer Solution: (30%T 2.7% C Bis) (37.5:1)

WARNING: Do this in a HOOD, be sure to wear GLOVES and GOGGLES.

To make 100 mL of solution, mix in a 200 mL beaker, using a magnetic stirrer:

Acrylamide solution (40% w/v) (very toxic)	75 mL
Distilled Water	25 mL
Bis-acrylamide 70/100 (very toxic)	0.8 g

Place in labeled container and store in refrigerator (NS105). This solution will keep for 1 to 2 months.

2. Resolving gel buffer (1.5M TRIS-Cl, pH 8.8)

To Make 100 mL of solution:
(this is enough for preparing 20 gels; two groups per gel because there are 10 wells per gel, eight groups per class, so 20 gels serves five classes.)

Tris	18.1 g
Distilled Water	80 mL

Titrate to pH 8.8 with 6M HCl using a Pasteur pipet (~12 ml). Add additional DI water to reach 100 mL final volume.

3. 10% w/v SDS Solution

To make 100 mL of solution, mix:

SDS (Sodium Dodecylsulfate - M.W.=228)	10 g
Distilled Water	100 mL

Be careful not to shake this solution, but rather stir

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it gently to prevent foaming. (SDS is a detergent)

4. 10% Ammonium Persulfate (Initiator of polymerization)
To make 5 mL of solution, in a 20 mL beaker with a microstirrer in the hood, mix:

Ammonium Persulfate	0.5 g
Distilled H ₂ O	5 mL

NOTE: THE ABOVE SOLUTION HAS NO SHELF LIFE, MUST BE MADE DAILY

5. Tank Buffer/Upper Chamber (cathode)
(0.025 M Tris, pH 8.3; 0.192 M glycine; 0.1% SDS)

To make 4 L of solution, mix:

Tris	12 g
Glycine	57.6 g
SDS	4.0 g
Distilled Water sufficient to make	4.0 L

6. Chamber Buffer/Lower Chamber (anode)
To make 2 L solution, mix:

Sodium Acetate	16.4 g
Tank Buffer	2 L

B. GEL RECIPES AND POURING INSTRUCTIONS FOR TEN 1.5 mm GELS

1. Making the gel solution:

a. Mix in a 250 mL Erlenmeyer flask with hose attachment:

Monomer Solution	50.0 mL (Very Toxic)
Resolving Gel Buffer	37.5 mL
10% SDS	1.5 mL
Distilled Water	60.0 mL

b. Degas the above mixture:

i. Place mixture with stir bar inside a vacuum chamber.

ii. Place vacuum chamber & beaker on a magnetic stirrer.

iii. Using a vacuum pump, pull a vacuum for 30 min.

NOTE: Gel casting chamber and Gel plates must be ready before

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proceeding with directions. They can be prepared during the thirty minute degasing period.

2. Preparing the gel chamber and trays:
 - c. Order the trays as follows:
 1. wax paper
 2. Glass
 3. gel space/spacers
 4. Alumina spacer (white)
 5. Repeat for all ten gels
 - d. Use stopcock grease (such as Thomas Lubriseal) around rubber gasket to seal gel chamber.
 - e. Place four clamps (two on each side) of the casting apparatus.
3. Casting gels:
 - f. After degasing, simultaneously ADD (as close together as possible):

Ammonium Persulfate (made that day)	750 μ L
TEMED	75 μ L
 - g. **IMMEDIATELY** after adding TEMED and Ammonium Persulfate, pour the gel solution into the gel trays, which are inside the casting block.
 - h. Add sec-Butyl Alcohol to the top of the gel solution to prevent air reaching the gel.
 - i. Place the combs properly into the gel solution.
 - j. Drop Sec-Butyl Alcohol over top of combs.
 - k. The gels take around 40 min. to solidify.
4. Removing gels from pouring chamber:
 - l. **Remove combs carefully !!**
 - m. Leave the glass plates and spacers in place.

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5. Storage:

- n. Place gel sandwich (gel plus glass, plastic spacers, and alumina plates) in air tight plastic container (such as RubberMaid Saver) with 5 mL resolving gel buffer, then refrigerate. SHELF LIFE OF GELS: 1 SEMESTER

C. PREPARING STOCK SOLUTIONS TO LOAD AND RUN SAMPLE

1. 2X Treatment Buffer : (0.125 M Tris-Cl, pH 6.8; 4% SDS; 20% glycerol; 10% 2-mercaptoethanol)

Use fume hood !!! 2-Mercaptoethanol is toxic and VERY offensive.

0.125M Tris-Cl	2.5 mL
4% SDS	4.0 mL
20% Glycerol	2.0 mL
2-mercaptoethanol	1.0 mL
Distilled H ₂ O	0.5 mL

2. 5X Ficoll Loading Buffer - Tracking Dye - Blue Color

Ficoll (w/v)	2.5 g
Tris Base	.32 g
EDTA (free acid)	.219 g
Bromophenol Blue	.01 g
Distilled H ₂ O	to make 10 mL total volume

3. .25% Coomassie Blue Stain R-250

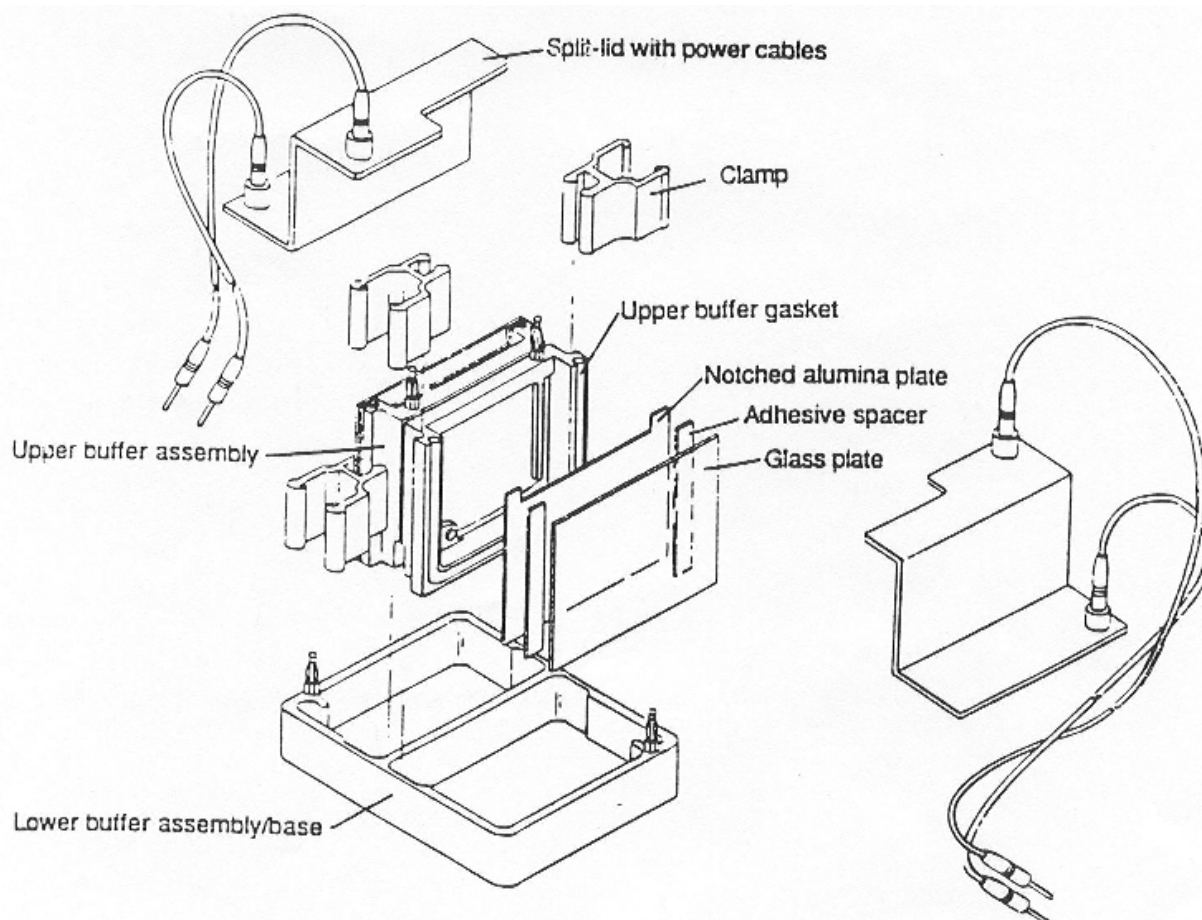
Coomassie Blue R-250	1.25 g
Methanol	200 mL
Glacial Acetic Acid	50 mL
Distilled H ₂ O	250 mL
Stir and Filter	

4. Destaining Solution (40% methanol, 10% acetic acid)
To Make 1 Liter:

Methanol	400 mL
Acetic Acid	100 mL
Distilled Water	500 mL

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- D. DIRECTIONS FOR SETTING UP APPARATUS WITH THE BUFFER SOLUTIONS (see drawing below):



Fisher Biotech Dual Mini Vertical Electrophoresis System

1. Coat gaskets of gel apparatus with stopcock grease.
2. Place one gel sandwich on each side, fastening with clamps.
3. Fill upper chamber with tank buffer. Watch for leaks into lower tank. If there is leaking, remove the gel and regrease the gaskets.
4. Fill lower chamber with chamber buffer (containing sodium acetate).
5. Set aside while samples are prepared.

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E. PROCEDURE FOR PREPARING FISH SAMPLES.

See the Student Laboratory Procedure on Page 2.

F. LOADING AND RUNNING SAMPLES.

1. Load gels with 30 μ L of each sample into its individual well, using the special gel loader pipet tips.
2. Be sure buffer solution completely covers gels.
3. Place top of apparatus on and place electrodes in the proper position.
4. Set the voltage meter at 80 v.
5. Run for approximately 45 to 60 minutes.
6. Check after 40 minutes to make sure that the sample is not running off the bottom of the gel.
7. When samples have traveled to within one inch of the plate, stop the electrophoresis process.

G. UNLOADING AND STAINING THE GEL

1. Remove electrodes and disconnect the apparatus from the electrical current.
2. Disconnect the POWER SUPPLY.
3. Remove the gel sandwich from the setup and carefully take the sandwich apart. Slip a flat surface under the gel, being careful not to break the gel.
4. Slide gel into large weigh boat and add staining solution. [see prep notes for stain recipe]
5. Leave in staining solution for two hours on shaking table. After staining, return staining solution to original container for reuse.
6. Place gel into destaining solution and return to the shaking table.

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7. After thirty minutes, pour the destaining solution down the drain, replace with fresh destaining solution and return the gel to the shaking table. Continue to shake until gels are clear, replacing the destaining solution one more time if necessary. Destaining can take overnight.
8. Place gels in ziplock bags and examine the protein bands on the light box.
9. Photos of the gels may be taken with black & white Polaroid film. Settings: F16/125