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Title: SDS-PAGE Electrophoresis of GFP

Approvals:
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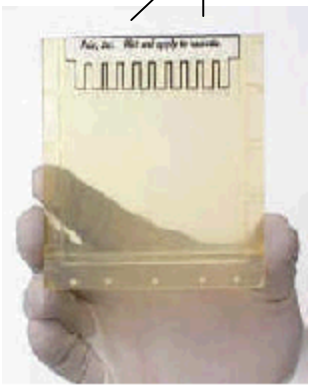
1. **Purpose:**
   1.1. To describe the appropriate operating instructions to perform SDS PAGE analysis of proteins samples.
2. **Scope:**
   2.1. Applies to confirming the presence and purity of the green fluorsecent protein (GFP) we have produced and purified in this class.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Gel box instruction instructions
   4.2. Gel Documentation Instrument SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Acrylamide is a neurotoxin. Always wear protective gloves when handling the polyacrylamide gels.
   6.2. Fixative solution is acidic and flammable – keep away from sparks and flames. Dispose in Fixative Hazardous Waste bottle
   6.3. Comassie Brilliant Blue stain is harmful. Handle carefully and dispose in hazardous waste bottle.
7. **Materials**
   7.1 Vertical electrophoresis box (see example below)
   7.2 Power Supply, 0-300 Volts
   7.3 Pre-poured Polyacrylamide Gels prepared with 4-15% polyacrylamide in Tris-HCl, 10cm x 10cm
   7.4 Pipette 20µl, 200µl
   7.5 Pipette tips
   7.6 Tris-Glycine Running Buffer, 0.05 M pH 8.8
   7.7 2X Laemmli Sample Buffer, including glycerol, bromophenol blue tracking dye and BME (or DTT:154 mg/10ml of sample buffer for entire class)
   7.8 Boiling water bath and microfuge holders
   7.9 Protein standards

7.10 Staining trays
7.11 Coomassie Brilliant Blue Stain
7.12 10 microfuge tubes
7.13 Lab marker pen, kimwipes
7.14 (Optional) Bio-Rad P6 Desalting Spin Columns

Sample Wells



Assorted PAGE Gel Boxes

8. Procedure:

8.1 Gather the samples and GFP positive fractions to test including:
- 8.1.1 Bacterial lysate supernatant.
- 8.1.2 HIC column microfuge tube fractions 1,2,3.
- 8.1.3 IEX (Anion) Exchange microfuge tube eluted fractions 1,2,3,4,5,6
- 8.1.4 IEX (Cation) Exchange microfuge tube eluted fractions 1,2,3,4,5,6
- 8.1.5 Kaleidoscope MW protein ladder, 10 µl.
- 8.1.6 GFP standard diluted 5 x, then suspended in 2X laemmli buffer.

8.2 (Optional) Desalt HIC fractions using the P6 Spin Columns:
- 8.2.1 After resuspending the gel in P6, snap off the tip of the column and place it in a 2.0 ml microfuge tube. Allow buffer to drain from column into tube.
- 8.2.2 Spin P6 Columns 2 min in microcentrifuge to remove buffer and discard the buffer.
- 8.2.3 Place the column(s) into a clean 1.5 ml centrifuge tube(s), pipette 50ul sample from HIC microfuge tubes 1, 2, and 3 into separate spin columns.
- 8.2.4 Spin columns 1 min and collect desalted HIC samples

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8.3 **Prepare your samples for electrophoresis.**

8.3.1. Locate the 20 µl in microfuge samples from the following sources
- 8.3.1. a Bacterial lysate supernatant
- 8.3.1 .b. HIC, fraction 2,3
- 8.3.1. c. Q IEX, fractions 2-6
- 8.3.1. d. S IEX, fractions 2,3

8.3.2 Make sure each sample from lysate and column fraction are carefully labeled.
8.3.2.Prepare samples: place 5 µl of 4X Laemmli buffer into each of 20 µl samples, place samples into boiling water bath for 3 minutes.
8.3.3. Prepare GFP standard: place 15µl of diluted GFP standard into a microfuge tube, add 5 µl of 4X Laemmli sample buffer, mix, boil.
8.3.4 Dilute 5 µl of Kaleidoscope protein standard by adding 20µl of H_20.
Note: Do not boil or add laemmli buffer

8.4 Assemble SDS-PAGE gel box according to SDS-PAGE Equipment SOP, Protein is Cash Manual, pages 53-56.

8.4.1 After assembling the gel box, take the gel out of the package and
☆ Make sure to remove green tape at the bottom of the gel.
8.4.2 Place gels into the box as instructed in SOP pg 56.
8.4.3 Rinse out gel wells with running buffer.
8.4.4 Place 5µl diluted with Kaleidoscope MW Protein Ladder, in lane 1.
8.4.5 Place 20µl of each sample into assigned well of the gel as shown below in the diagram.
8.4.6 Place 5µl of diluted GFP in lane 10.
8.4.7 If you have more than 8 samples from your collected fractions you will need to prepare a second gel to accommodate additional samples. If using a second gel, make sure to add both protein standard and GFP stds

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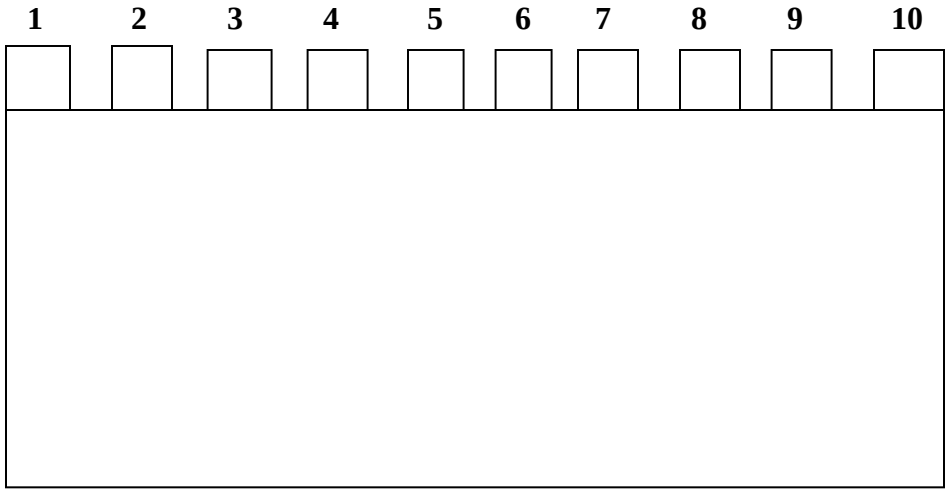
1 Kaleidoscope MW Protein Ladder (5µl)
2 Bacterial Lysate Supernatant (20ul for all samples 2-9)
3 Chromatography Sample
4 Chromatography Sample
5 Chromatography Sample
6 Chromatography Sample
7 Chromatography Sample
8 Chromatography Sample
9 Chromatography Sample
10 GFP standard (5 ul)



8.5 **DANGER: Care must be taken with high voltage.**

8.5.1 Wait for your instructor to assist you in turning on the Protean Tetra PAGE equipment.

8.5.2 Run gels at 200 volts for 30 minutes. **Do not allow running dye to go off the bottom of gel. Turn off electricity and unplug. Electrophoresis equipment before opening to take out PAGE gels.**

8.5.3 Remove gel from SDS-PAGE gel box and place in staining tray according to SOP Mini-Protean Tetra-Gel, p.53. Wash with distilled water 2x for 1-2 minutes, then pour off all water.

8.6 Visualizing the protein bands on SDS PAGE gel after Electrophoresis; Stain and De Staining Gels.

8.6.1 Pour Coomassie Brilliant Blue Stain over gel until it is completely covered with dye and stain them for 30 minutes while gently shaking.

8.6.2 Pour off stain and wash 2x in DI water then Pour off DI water.

8.6.3 Destain with Coomassie destain solution changing the solution 2-3 times. Place pieces of twisted kimwipes in destain solution with gel. Watch for the bands to appear, which usually occurs within 1 hour.

8.6.4 Photo-document gel or draw the bands on paper as shown on gel.

Results and Analysis of Protein Bands Electrophoresed by SDS PAGE

8.7 Procedure for determining the molecular weight of unknown proteins.

8.7.1 Running known proteins of known molecular weight which can be visualized on PAGE gels allows one to calculate the relative migration of the protein compared to the running dye front called the R_f value.

8.7.2 Figure 1 shows the standard stained proteins and their molecular weights as they appear on a 4-20% Tris-Glycine PAGE post electrophoresis and staining.

8.7.3 Rf value for each protein band is found by measuring how far the selected band moved compared to how far the running dye front moved from the beginning gel well. This forms a ratio of speed of the protein divided by the speed of the running dye.

Definition R_f = $\frac{\text{Distance from starting well to one protein band (cm)}}{\text{Distance from starting well to running dye front (cm)}}$

8.7.4 Table 1 contains the molecular weights in kiloDaltons and R_f values of the standard proteins used in the SDS PAGE activity done.

FIGURE 1
Standard Protein Bands on SDS PAGE

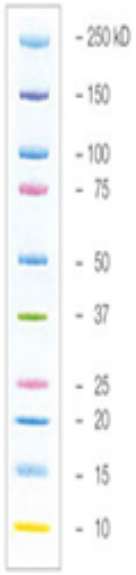


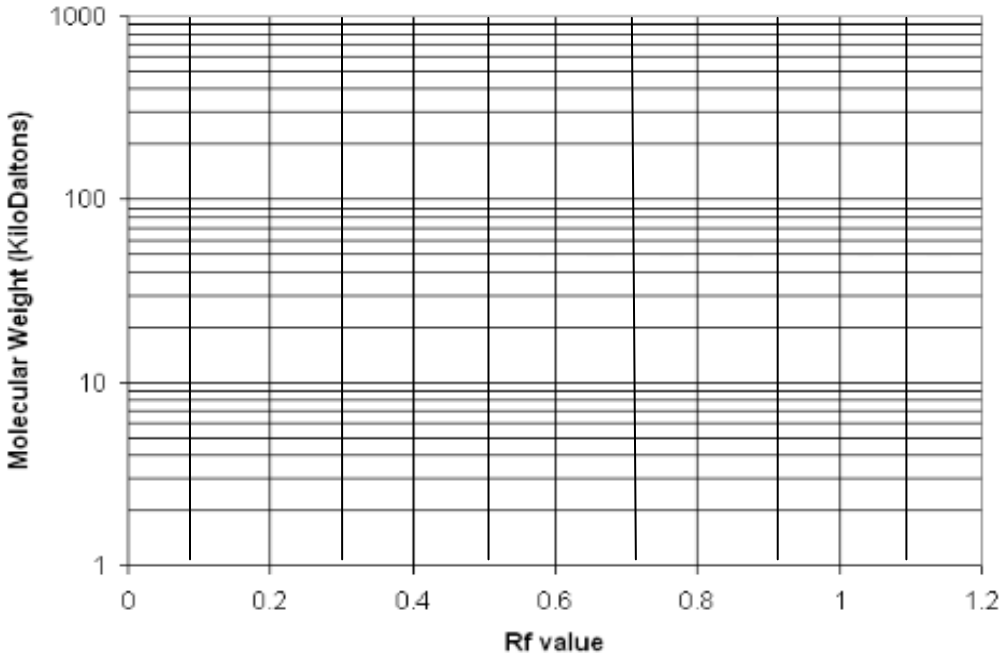
TABLE 1

Band Color	Molecular weight kD	R_f of band = Band to dye front in cm / gel top to dye front in cm
	y axis	x axis
blue	250	0.5/7.5 = 0.07
purple	150	1.2/7.5 = 0.16
blue	100	2.0/7.5 = 0.27
red	75	2.5/7.5 = 0.33
blue	50	3.4/7.5 = 0.45
green	37	4.2/7.5 = 0.56
red	25	5.1/7.5 = 0.68
blue	20	5.6/7.5 = 0.75
Lt blue	15	6.3/7.5 = 0.84
yellow	10	7.1/7.5 = 0.95
Dark blue	Unknown	4.8/7.5 = 0.64

Generate a plot using the semi-log graph shown below using the information in Table I.

8.8.1 The protein standard has two values, a molecular weight in kilo -Daltons found on the y axis and the Rf, found on the x axis. Find the point on a semi-log graph for the first standard protein, X, Y, determining one position on the graph.
8.8.2 Repeat the same process as in 9.2.1 for each known protein until all the protein are plotted on the semi-log graph.
8.8.3 Draw a best fit straight line with a ruler, using the points on the graph.

FIGURE 2
STANDARD CURVE of
SDS PAGE: PROTEINS



8.9 Finding the molecular weight of an unknown protein using a standard graph created in 8.8.3.
8.9.1 Calculate the R_f of the unknown protein by determining the band distance migrated in cm of the unknown divided by the distance of the running dye in cm as shown in Figure 1.

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8.9.2 Once the R_f is calculated for the unknown protein.
refer to the standard graph plotted as molecular weight vs R_f.
8.9.3 Find the R_f value on the x axis and draw a line vertically that will intersect with the line on the graph, the point that it crosses the line given for standard proteins .
8.9.4 Draw a line horizontally from this x axis to the line on the graph. the y axis for the point intersected on the y axis is its molecular weight.
8.10 SDS PAGE pGLO transformed E.Coli lysate, and post column Purification.

FIGURE 3

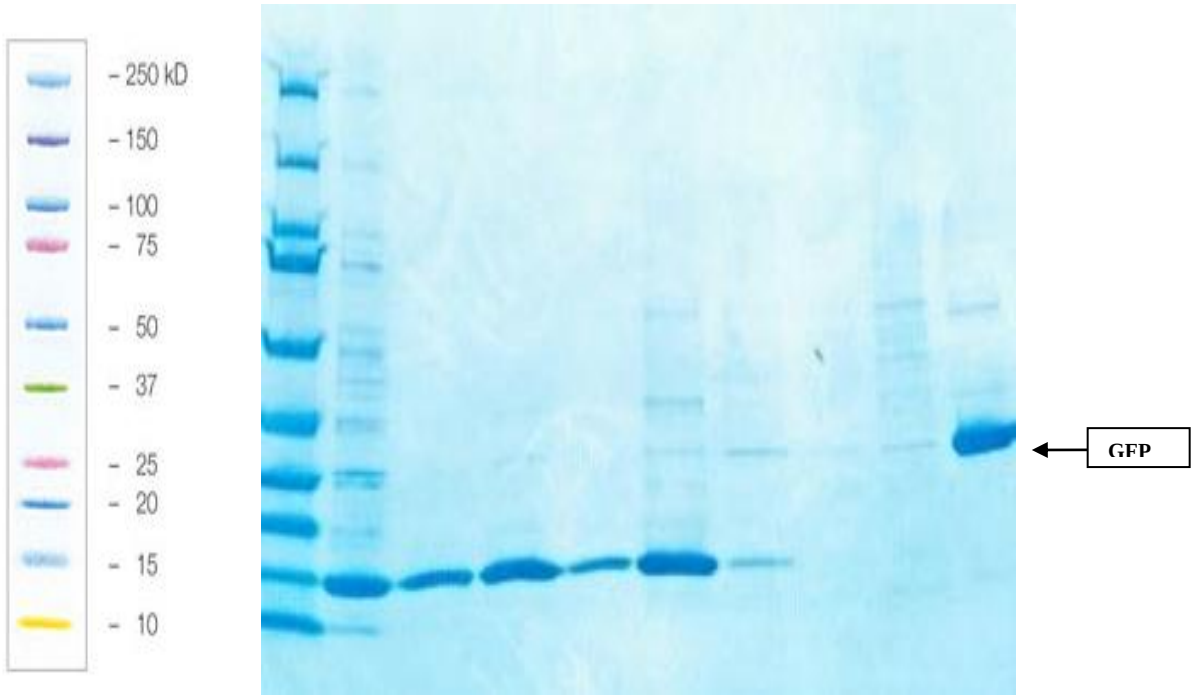

Figure 3: PAGE SDS electrophoresis of cell lysate, HIC column eluates fractions 2,3, Q column eluate fraction 2 (0.13M NaCl + buffer), Q eluate column fraction 4 (0.30M NaCl + buffer), Q eluate column fraction 6 (0.5M NaCl + buffer), S column eluate fraction 2 (0.13M NaCl), S column fraction 4 (0.30M NaCl + Buffer) and GFP std indicated with arrow (last lane). note: standard proteins may have bled into the cell lysate protein lane.

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8.11 Determine which lane(s) on this PAGE gel shown above has a unknown protein band that equals the Rf of green fluorescent protein, (GFP, molecular weight 27.5 kilo Daltons). There is some distortion due to heating the gel during the electrophoresis.