SDS/PAGE MINI PROTEIN GEL

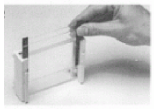
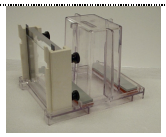
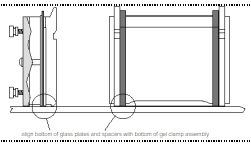
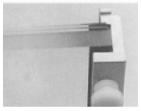
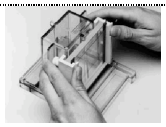
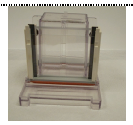
Polyacrylamide gel electrophoresis (PAGE) is a widely used technique for separating proteins. The most widely used method was developed by Laemmli (Nature 227: 690-685, 1970) using the denaturing (SDS) discontinuous method. This protocol relies on the presence of SDS (sodium dodecyl sulfate) and ß-mercaptoethanol to denature the proteins, dissociate the proteins into subunits, and to coat them with negative charges (SDS). This relatively uniform charge to mass ratio allows the proteins to migrate in an electric field and separate according to mass/size. The Laemmli system uses buffers of different pH and composition to generate a voltage gradient and a discontinuous pH between the stacking and the resolving gel. A 4% acrylamide stacking gel (pH 6.8) is poured on top of a 10% acrylamide resolving gel (pH 8.8). The stacking gel (with a large pore size) serves to concentrate all of the proteins (the large ones can catch up with the small ones) on top of the resolving gel. After entering the resolving gel (which has a smaller pore size) the proteins are separated according to relative molecular size.

Protein samples are diluted 1:2 in Laemmli sample buffer and are boiled for 5 minutes. The ß-mercaptoethanol in the sample buffer reduces the protein's disulfide bonds and the SDS denatures the proteins. The sample buffer contains glycerol to increase the density so that when the sample is loaded it sinks to the bottom of the well. Bromophenol blue dye is in the sample buffer to monitor the electrophoresis process. The gel electrode assembly is placed in the Mini PROTEAN II electrophoresis chamber (BIORAD), reservoir buffer is added to upper and lower chambers, the samples are loaded and the proteins electrophoresed at ~200Volts for about 45 minutes.

For proteins 30-90kD using an 8-10% gel, the bromophenol blue dye should travel to the bottom of the resolving gel. The gel can then be used to transfer the proteins to nitrocellulose for a Western or can be fixed, stained in Coomassie blue dye and destained. For detection of proteins in the gel with Coomassie blue (a standard stain for total protein), at least 1 µg of protein per band is required.

Reagents	Materials
1.5M Tris pH 8.8	BioRad Protean II Mini gel unit and casting stand
0.5 M Tris pH 6.8	per gel:
30% Acrylamide	2 spacers (0.75 or 1.5mmm)
dH_2O	1 large glass plate (10.2x8.3mm)
10% SDS	1 small glass plate (10.2x7.3mm)
TEMED	1 comb (10 or 15-well) (0.75 or 1.5mmm)
10% APS	optional: "spacer-mate" plate alignment card
1X Reservoir buffer	
Laemmli sample buffer	note: be consistent with 0.75mm or 1.5mm

BioRad SDS/PAGE Mini Protean II Gel: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1. Assemble the BioRad Protean II Mini gel on a clean surface. Lay the longer rectangular glass plate down first, then place two spacers of equal thickness along the short edges of the rectangular plate. Next, place the shorter glass plate on top of the spacers so that the bottom ends of the spacers and glass plates are aligned. At this point, the spacers should be sticking up about 5 mm above the long glass plate.
2. Loosen the four screws on the clamp assembly and stand it up so that the screws are facing away from you. Firmly grasp the glass plates with the spacers between them (with the longer plate facing away from you) and gently slide it into the clamp assembly along the front face of the acrylic pressure plate. The longer glass plate should be against the acrylic pressure plate of the clamp assembly. Tighten the top two screws of the clamp assembly.
3. Place the clamp assembly into the alignment slot on the casting stand so that the clamp screws face away from you. Loosen the top two screws to allow the plates and spacers to settle against the casting stand base. Insert the Mini-PROTEAN II "spacer-mate" alignment card between the glass plates in order to position the spacers properly. Gently tighten both pairs of screws.
4. Remove the alignment card. Remove the clamp assembly from the alignment slot of the casting stand. Check that the plates and spacers are flush at the bottom (important!). If not, re-align the plates and spacers again (steps 1-3).
5. Check to see that the removable gray silicone gaskets are clean and free of any residual acrylamide to insure a good seal.
6. Transfer the clamp assembly to one of the casting slots in the casting stand. If two gels are to be cast, place the clamp assembly on the side opposite the alignment slot to allow space to assemble the second set of gel plates.
7. Put the acrylic pressure plate against the wall of the casting slot at the bottom, so the glass plates rest on the rubber gasket. Snap the acrylic plate underneath the overhang of the casting slot by pushing with the white portions of the clamps. Do not push against the glass plates or spacers. This could break the plate.

Helpful suggestions:

- It is recommended to fill the assembled cassette first partially with water, marking the meniscus with a felt tip pen. If no leakage is detected in 5 minutes, pour out the water and remove the residual water by inserting a filter paper. You can then begin to pour the gel.
- It is sometimes useful to place a double-layer of flat, smooth parafilm on top of the gray gasket of the casting stand to keep the unpolymerized gel from leaking.

Pouring the Gel:

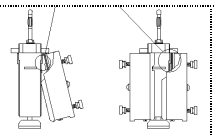
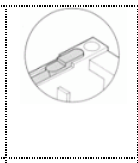
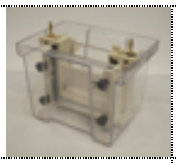
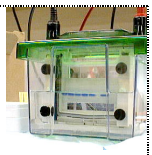
1. Prepare the resolving gel according to the recipe. It is useful to add all the reagents except the TEMED to the gel (once the TEMED and APS have been added to the polyacrylamide solution will polymerize in a few minutes).
2. to pour the gel, use either:

p-1000		plastic 10 ml disposable pipette	
set volume at 880 µl and add that volume		Pour resolving gel up to ~ 2 cm from top	
0.75mm	1.5mm	0.75mm	1.5mm
4x 880 µl	8x 880 µl	3.5ml	7ml

3. Immediately after pouring the gel, gently add a 1-2 ml layer of dH_20 on top of the gel to avoid exposure to air. Leave the gel to polymerize (typically 5-15min). A sharp line between water layer and gel indicates completion of polymerization. While waiting for the gel to polymerize you can start preparing the stacking gel.
4. When the polymerization of resolving gel is complete, remove the layer of water (tip unit upside down and collect the dH_20 using Kim-wipes.
5. Pour the stacking gel directly on top of the resolving gel using a pasture pipette or P-1000 to the top of the large glass plate. Insert the comb gently and quickly before the gel polymerizes. Leave to polymerize until gel gets firm (generally about 10 min).

Assembling the Electrophoresis unit:



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1. Release the glass plate/clamp assembly from the casting stand and lay the inner cooling core down flat on a lab bench.
2. With the glass plates of the gel plate assembly facing the cooling core (and the clamp screws facing out), carefully slide the clamp assembly wedges underneath the locator slots on the inner cooling core until the inner glass plate of the gel assembly butts up against the notch in the U-shaped gasket.
3. To insure a leak-proof seal, make sure that the gray, V-shaped, inner core gaskets are clean and that they are installed with the notched side exposed for contact with the gel plate. *Note:* wetting the gasket with a drop of running buffer or water helps the glass plate sandwich slide in properly.
4. Put the mini-protein gel/electrode assembly in the electrophoresis chamber. Fill the inner chamber to the top with 1xReservoir Buffer/0.1%SDS. Pour additional 1xReservoir Buffer/0.1%SDS into the outer chamber until the level is above the bottom of the glass plate. Note: If the inner buffer chamber is leaking to the outer chamber, fill the outer chamber up to the top of the glass plates.
5. Load the gel using the long gel-loading tips or a Hamilton Syringe.

6. Connect the red and black electrical leads in the green cover to the matching colored electrodes of the electrode assembly. Plug in the electrodes into the matching color receptacles on the power supply (note the red ring around the anode and black ring around the cathode of the electrode assembly).
7. We typically run the proteins through the stacking gel at 80-100V, then turn up the power and increase the voltage until the display reads ~200 V (approximately 60 mA) for one gel. An outpouring of bubbles indicates a good electrical connection. Run the gel about 30-45 minutes, until the bromophenol blue dye has migrated to the bottom of the gel.
8. When finished: remove the electrode assembly from the buffer tank and pour the running buffer down the sink. Lay the electrode assembly flat on a surface with the protein gel facing you. Carefully remove the glass plates from the electrode assembly by gently pulling the bottom corners of the assembly down and pushing up on the clamps until the clamp assembly is released.
9. Loosen all four screws of the clamp assembly and remove the glass plate assembly. Remove one of the gray spacers from the assembly. Remove the other spacer part way and use it to gently twist apart the glass plates. The gel should remain stuck to one of the glass plates.

Helpful suggestions:
It is especially important to assure that the rubber gasket is placed correctly (with notch facing glass plate), and that the bottom is aligned exactly to give a smooth seal.

SDS/PAGE Mini Protein Gel recipes

10% Separating 0.75 mm Gel:

	1 gel	2 gels	3 gels	4 gels	5 gels	6 gels
dH_2O	1.9 ml	4.0 ml	6.0 ml	8.0 ml	9.9 ml	11.9 ml
1.5 M Tris pH 8.8	1.3 ml	2.5 ml	3.8 ml	5.0 ml	6.3 ml	7.5 ml
30% Acrylamide	1.7 ml	3.3 ml	5.0 ml	6.7 ml	8.3 ml	10.0 ml
10% SDS	50 µl	100 µl	150 µl	200 µl	250 µl	300 µl
10% APS	50 µl	100 µl	150 µl	200 µl	250 µl	300 µl
TEMED	2 µl	4 µl	6 µl	8 µl	10 µl	12 µl

5%Stacking Gel:

	1 gel	2 gels	3 gels	4 gels
dH_2O	1.4 ml	2.7 ml	4.1 ml	5.5 ml
1.0 M Tris pH 6.8	250 µl	500 □l	750 □l	1.0 ml
30% Acrylamide	330 µl	670 µl	1.0 ml	1.3 ml
10% SDS	20 µl	40 µl	60 µl	80 µl
10% APS	20 µl	40 µl	60 µl	80 µl
TEMED	2 µl	4 µl	6 µl	8 µl

Various Percentage Gels:

for 1 gel:	6 %	8 %	12 %	15 %
dH_2O	2.6 ml	2.3 ml	1.6 ml	1.1 ml
1.5 M Tris pH 8.8	1.3 ml	1.3 ml	1.3 ml	1.3 ml
30% Acrylamide	1.0 ml	1.3 ml	2.0 ml	2.5 ml
10% SDS	50 µl	50 µl	50µl	50µl
10% APS	50 µl	50 µl	50µl	50µl
TEMED	4 µl	3 µl	2µl	2µl

Recipes for SDS/PAGE gels and buffers					
30% Acrylamide	100ml:	500ml:			
	29g	145 g	2X Acyrlamide		
	1g	5 g	*N,N'*-methylenebisacrylamide		
	Add ~300ml dH_2O (heat to 37°C to dissolve chemicals). Adjust final volume to 500ml with dH_2O. Sterilize by filtration through a Millipore 0.22µm vacuum filter. Store in the dark at 4°C for 1 year. *Acrylamide is a potent neurotoxin and can be absorbed through the skin. Wear gloves and a mask when weighing the reagents. Wear gloves when handling the solutions containing these reagents.*				
10% APS Ammonium persulfate	10ml: 1g Ammonium persulfate Add dH_2O to 10ml This solution may be stored at 4°C for several weeks.				
Laemmli sample buffer (SDS reducing buffer)	2X Laemmli sample buffer			5X Laemmli sample buffer	
	16ml:			16ml:	
	10.4	dH_2O		6.8ml	dH_2O
	1.2 ml	0.5M Tris pH 6.8		2.0 ml	0.5M Tris pH 6.8
	1.9 ml	Glycerol		3.2 ml	Glycerol
	1.0 ml	20% SDS		1.6 ml	20% SDS
	0. 5ml	ß-mercaptoethanol		0.8 ml	ß-mercaptoethanol
		1% bromophenol blue		1.6 ml	1% bromophenol blue
	Store in aliquots at –20°C Dilute with sample 1:4 and boil 5 min.				
10X Reservoir Buffer	1 liter	2 liters:			
	30 g	60 g	Tris base		
	144 g	288 g	glycine		
	to 1 liter	to 2 liters	dH_2O		

	add 5 ml/liter 20% SDS for electrophoresis
10% SDS	100ml: 10g SDS (Sodium dodecyl sulfate) add dH_2O to ~90ml heat ~60°C to facilitate dissolving No need to sterilize.
0.5M Tris pH 6.8	500ml: 30.275 g Tris Base add dH_2O to ~400 ml Adjust pH to 6.8 with conc. HCl Bring up volume to 500 ml
1.5 M Tris pH 8.8	500ml: 90.825 Tris Base add dH_2O to ~400 ml Adjust pH to 8.8 with conc. HCl Bring up volume to 500 ml

References:

Current Protocols in Molecular Biology, eds. F.M. Ausable, et al. Wiley Press, N.Y., 1989.

BIORAD instruction manual for Mini-PROTEAN II.

Laemmli, U.K., Nature, 227, 680, (1970).