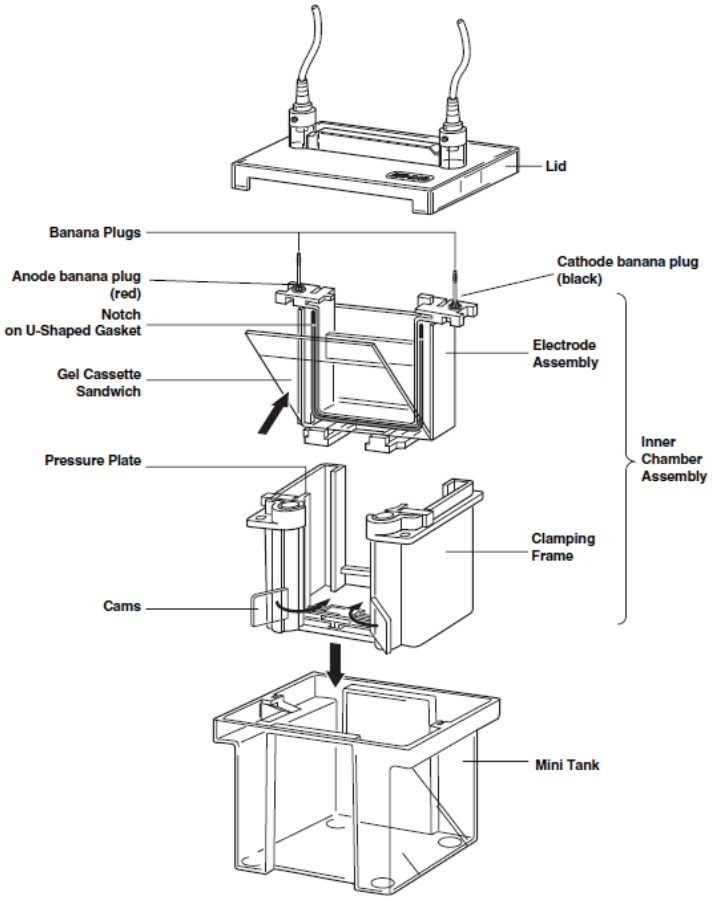
Protocol BioRad SDS-PAGE Gel Electrophoresis



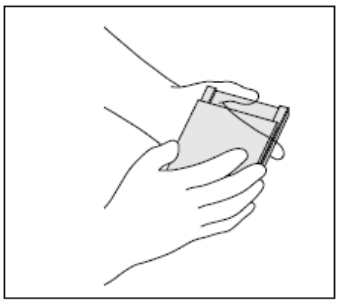
WEAR GLOVES AT ALL TIMES

ACCYLAMIDE IS NEUROTOXIC

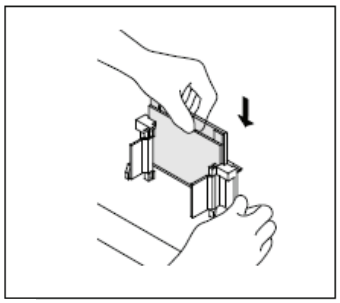
1. For one gel, clean a Short Plate and a Spacer Plate (taller, with "spacer" bars at the sides, 0.75/1.0/1.5 mm thick) using lint-free tissue (e.g., Kim Wipes):
   (a) with MilliQ water
   (b) with 70% EtOH.
   Dry the plates with tissue and place them on clean bench paper (don't touch with fingers).

2. On a clean, level surface (a clean bench), place a Casting Frame (green) with the pressure cams facing forward.

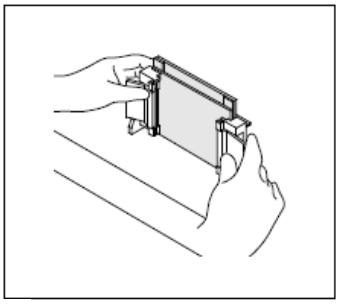
   (a) Place the Short Plate on top of the Spacer Plate to create a Gel Cassette (see left figure below).
   (b) With the Spacer Plate labelling "up", slide the two glass plates into the Casting Frame, with the Short Plate facing forward, (see middle figure below).
   (c) Secure the Gel Cassette in the Casting Frame by rotating the pressure cams outward (see right figure below), while making sure that the glass plates are *FLUSH at the bottom* with each other and with the bench surface (otherwise leaking will occur).



Place a Short Plate on top of the Spacer Plate.

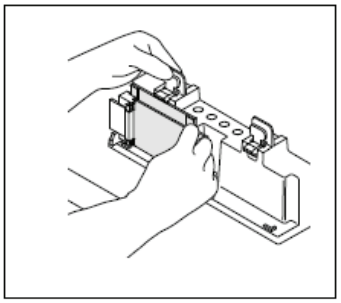


Slide the two plates into the Casting Frame keeping the Short Plate facing front.



Lock the pressure cams to secure the glass plates.

3. Secure the Casting Frame/Gel Cassette assembly on the Casting Stand (see figure below): Engage the spring-loaded lever, place the assembly on the grey gasket (make sure this is clean) with the back of the Casting Frame tightly placed against the Casting Stand, and release the lever. The lever pushes the glass plates onto the gasket, making a seal if they are flush.

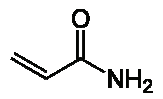


Secure the Casting Frame in the Casting Stand by engaging the spring loaded lever.

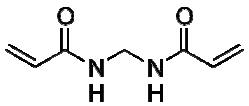
4. Insert a clean comb completely into the assembled Gel Cassette, and mark the glass plate 1 cm below the comb teeth (level to which Resolving Gel is poured). Remove the comb.

5. In a 15-ml Greiner tube, prepare the **RESOLVING GEL SOLUTION** by combining:

MilliQ water	1670 µl	
4*RB	1000 µl	*RB = Resolving Buffer*
30% AA	1330 µl	*AA = (mono-)acrylamide & bis-acrylamide (37.5:1)*



(mono-)acrylamide

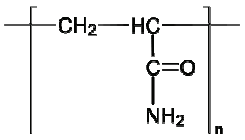


bis-acrylamide

Mix gently by inverting the tube 3 times (don't vortex: air bubbles!), then add (in this order):

10% APS	20 µl	APS = Ammonium persulfate, $(NH_4)_2S_2O_8$
TEMED	2 µl	TEMED = N,N,N′,N′-Tetramethylethylenediamine

Mix quickly, but *gently*, by inverting three times (no vortexing: air bubbles!).
This starts the polymerisation reaction, so the solution should be used immediately.



polyacrylamide

6. Using a blue tip, or a 5-ml serological pipette, gently pipette the solution into the Gel Cassette on the Casting Stand: hold the tip against one of the spacers and refrain from harsh pipetting. Fill the Gel Cassette up to the mark you made in step 4.

7. Immediately overlay the Resolving Gel solution with ~ 1 ml of MilliQ water: pipette *gently* via one of the spacers. Prevent mixing the water and gel solution phases by harsh pipetting.

   Allow the gel to polymerise for 30-45 minutes.

8. Remove the water phase from the polymerised gel (decant, and remove water remaining between the glass plates of the Gel Cassette with a piece of clean Whatman paper while making sure not to touch the gel).

9. In a 15-ml Greiner tube, prepare the **STACKING GEL SOLUTION** by combining:

MilliQ water	1180 µl	
4*SB	500 µl	*SB = Stacking Buffer*
30% AA	320 µl	*AA = (mono-)acrylamide & bis-acrylamide (37.5:1)*

Mix gently by inverting the tube 3 times (don't vortex: air bubbles!), then add (in this order):

10% APS	10 µl	*APS = Ammonium persulfate, $(NH_4)_2S_2O_8$*
TEMED	2 µl	*TEMED = N,N,N',N'-Tetramethylethylenediamine*

Mix quickly, but *gently*, by inverting three times (no vortexing: air bubbles!).
This starts the polymerisation reaction, so the solution should be used immediately.

10. Using a blue tip, gently pipette the Stacking Gel solution into the remaining space between the glass plates of the Gel Cassette, on top of the polymerised Resolving Gel: hold the tip against one of the spacers and refrain from harsh pipetting.
Fill the Gel Cassette up to the level of top of the Short Plate.

11. *Immediately* insert the comb by holding it at an angle, and inserting comb well 1 first, then well 2, then well 3, and so on, until the comb is completely inserted (some gel solution may spill over; that is OK, but be sure to wear gloves!).
Make sure the comb ridge is aligned with the top of the Short Plate, and that there are *no air bubbles underneath the comb teeth* (wells).

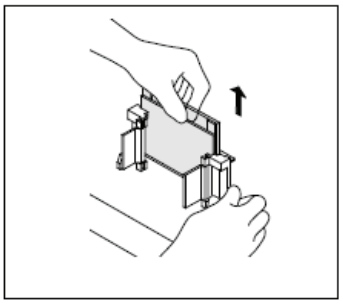
Allow the gel to polymerise for 10-15 minutes.

12. Prepare the **RUNNING BUFFER** by combining and gently mixing:

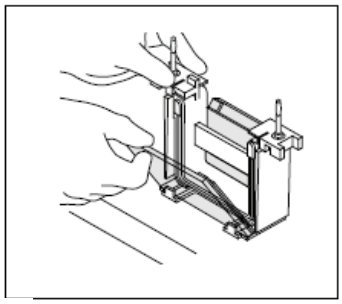
10*TGS	80 ml	*TGS = Tris-Glycine-SDS*
MilliQ water	720 ml	

13. Gently remove the comb from the polymerised Stacking Gel, and *immediately* rinse the wells with Running Buffer using a blue tip. Rinse ~ 3 times to remove unpolymerised acrylamide.

14. Remove the Gel Cassette from the Casting Stand. Rotate the cams of the Casting Frame inward to release the Gel Cassette (see left figure below).



Remove the Gel Cassette Sandwich from the Casting Frame.

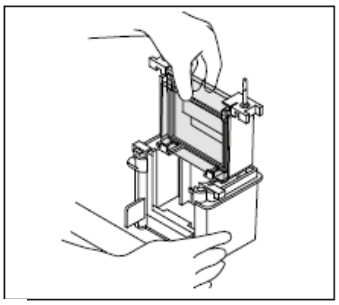


Place Gel Cassette Sandwich into the Electrode Assembly with the Short Plate facing inward.

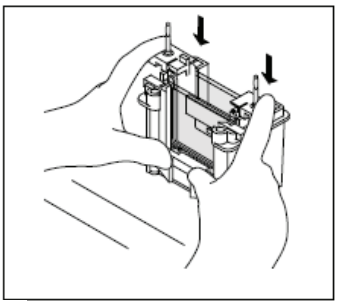
15. Place a Gel Cassette into the slot at the bottom of one side of the Electrode Assembly, with the Short Plate facing *inward* toward the U-shaped gaskets (see right figure above).

16. Do the same at the other side of the Electrode Assembly with either another Gel Cassette (if you will run two gels at the same time) or with a "Buffer Dam" (a Gel Cassette-sized piece of plastic).

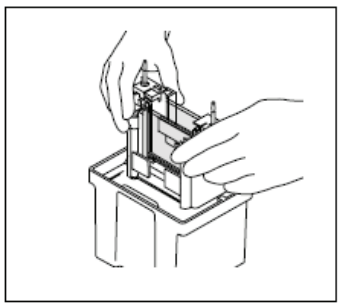
17. At both sides of the Electrode Assembly, bring the Gel Cassette(s)/Buffer Dam into full contact with the green gaskets, and slide this whole assembly into the Clamping Frame with the two cam levers of the latter facing forward (see left figure below).



Slide Gel Cassette Sandwiches and Electrode Assembly into the clamping frame.



Press down on the Electrode Assembly while closing the two cam levers of the Clamping Frame.



Lower the Inner Chamber into the Mini Tank.

18. *While pressing down on the Electrode Assembly*, rotate the cam levers of the Clamping Frame to form and secure the Inner Chamber, ensuring a proper seal of the top of the Short Plate(s) against the U-shaped gasket to prevent leaking (see middle figure above).

19. Lower the Inner Chamber assembly into the Mini Tank (see right figure above), and fill the Inner Chamber with Running Buffer (~125 ml) until the buffer level reaches halfway between the tops of the Spacer and Short Plates of the Gel Cassette(s). Do *not* overfill (will cause siphoning of buffer).

20. Fill the rest of the Mini Tank (the "outer chamber" or "lower chamber") with Running Buffer to ~ the same level as the level in the Inner Chamber.

21. *Have your samples ready by this time.* They should have been denatured by heating at 95-99 ^{o}C for 3-5 min, and spun in a microcentrifuge (5-10 min at full speed) to pellet particulates.

22. Check if there are no air bubbles in the wells of the gel(s); if necessary rinse with some buffer from the Inner Chamber compartment using a blue tip (or a syringe with a needle).

23. Load your samples with a Gel Loader tip: lower the tip in a selected well, and gently pipette out the sample. *Beware of air bubbles in the tip that will cause whirling in the well if pipetted out.*

    Always take along a (pre-stained) molecular weight (MW) marker in one of the wells.

24. Place the Lid on the Mini Tank. Make sure to align the (red/black) colour-coded banana plugs and jacks. Insert the electrical leads into a power supply.

25. Apply power and begin electrophoresis:
    (a) Start at **100 V** (constant voltage) until the blue dye front has traversed the Stacking Gel and reached the Resolving Gel (pre-stained MW marker will start to seprate).
    (b) Continue at **200 V** (constant voltage) until the dye front has reached the bottom of the gel.

26. Stop electrophoresis by turning off the power supply, and disconnect the electrical leads. Remove the Mini Tank lid and, *in the sink*, lift out the Inner Chamber assembly, open the cams of the Clamping Frame, pull the Electrode Assembly out of the Clamping Frame, and remove the Gel Cassette(s).

27. Using the green, wedge-shaped, plastic Gel Releaser to pry the glass plates apart, remove the Short Plate from the Gel Cassette by gently separating the two glass plates.

28. Gently run the sharp edge of the Gel Releaser (or a razor blade) along each spacer to separate the gel from the spacers on the Spacer Plate.

    Carefully transfer the gel to a *clean, keratin-free* container (keratin free for in-gel digestion purposes) with ~ 50 ml Fixing Solution, and agitate gently on a rocking platform.

29. Continue with the OPL Staining Protocol.

Solutions

4*RB (0.45 μm-filtered):

1.5 mM Tris-HCl pH 8.8
0.4% SDS

4*SB (0.45 μm-filtered):

0.5 M Tris HCl pH 6.8
0.4% SDS

30% AA:

30% Acrylamide/Bis Solution, 37.5:1

[Bio-Rad cat. nr. 161-0158]

TEMED:

[Bio-Rad cat. nr. 161-0801]

10*TGS:

250 mM Tris
1920 mM Glycine
1% SDS pH 8.3

[Bio-Rad cat. nr. 161-0772]