

Casting 12 BioRad Mini SDS PAGE Gels

1. Assemble multicasting chamber. Make sure ALL plates and spacers are flush with bottom. Clean gasket and coat with a medium-thin layer of Vaseline to avoid leaks.
2. Set up gradient maker and peristaltic pump. Make the 8% and 16% gel solutions in each side of gradient maker using two stir bars for mixing. No need to degas. Make fresh ammonium persulfate (25% APS = 0.25 g APS + 0.85 ml ddH₂O). Make sure valve and tube to chamber are not clogged.

	<u>8%</u>	<u>16%</u>
5X 8.8 Buffer	5.5 ml	5.5 ml
30% Acrylamide	7.5 ml	14.5 ml
ddH ₂ O	14.5 ml	2 ml
50% Glycerol	--	5.5 ml
TEMED	28 μl	28 μl
25% APS	35 μl	35 μl

3. After adding APS with stirring, open *end* and *middle* valves. Pump at speed "8" to pour gels (~10 min). Stop pumping when resolving gel is 1 cm below where comb teeth will be. Do not pump air past valve at bottom of multicasting chamber. Overlay each gel with 0.15 ml butanol (ddH₂O sat).
4. After polymerization (~30 min), wash with ddH₂O 3-5 times. Dry with triangles of filter paper.
5. Prepare stacking gel. Degas with vacuum aspirator. Use APS made above.

	<u>4.5%</u>
5X 6.8 Buffer	5 ml
50% Sucrose	5 ml
30% Acrylamide	3.8 ml
ddH ₂ O	11.2 ml
TEMED	25 μl
25% APS	25 μl

6. Introduce cleaned, dry combs. Place chamber at slight angle. Add stacker along lower side between spacers. Stacker will flow between gels. Place on flat surface. Finish adding stacker. Move comb with forceps from side to side to remove bubbles. Keep bottom of comb flush with top of spacers.
7. After polymerization, disassemble casting chamber. Carefully separate gels. Rinse with ddH₂O. Remove excess polyacrylamide. Place gels in zip-lock bags, place in plastic tray, and store at 4°C.

