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Making SDS-PAGE gels—Laemmli using 40% Acrylamide

Revised by J Sims
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Procedure

1. Clean glass plates off with EtOH
2. Measure 1.5 cm from top of shorter glass plate and mark on the plate
3. Set up gel stands and clamps
4. Add resolving gel solution up to the line (make resolving gel solution fresh after setting up gel stands)
5. Add water to the top of the short glass plate and let polymerize
6. Pour off water and carefully remove all residual water with a piece of Whatman paper.
7. Add stacking gel solution and combs. Let polymerize. (make stacking gel solution fresh after resolving gel solution has polymerized.)
8. Store gels wrapped in a damp paper towel and saran wrap at 4oC (good up to 1 month)

Solutions

Resolving gel	6 gels		3 gels	
Final % Acrylamide	40% acrylamide (mL)	water (mL)	40% acrylamide (mL)	water (mL)
5	2.75	10.85	1.375	5.425
6	3.3	10.3	1.65	5.15
7.5	4.125	9.475	2.063	4.738
8	4.4	9.2	2.2	4.6
10	5.5	8.1	2.75	4.05
12	6.6	7	3.3	3.5
15	8.25	5.35	4.125	2.675
22	12.1	1.5	6.050	0.75

Other components	6 gels	3 gels
1M Tris, pH 8.8	8.2 mL	4.1 mL
20% SDS	100 μ L	50 μ L
TEMED	10 μ L	5 μ L
10% APS	150 μ L	75 μ L

Stacking Gel	6 gels
40% acrylamide	0.50 mL
water	3.79 mL
1M Tris pH 6.8	0.63mL
20% SDS	25 μ L
TEMED	5 μ L
10% APS	50 μ L