

MINI-PROTEIN GELS

1. Clean plates with EtOH and set up in stand:

- make sure to have base clean
- keep plates to back of stand
- push down after clicking in plates
- mark where the stacking gel will be (1cm lower than the comb)

2. Make up gel solution as follows. Makes up enough for two gels.

	15%	12%	10%
H ₂ O	4.25 ml	5.0 ml	5.5 ml
2 M Tris pH8.8	1.8 ml		
10% SDS	100 μ l		
40% acrylamide	3.75 ml	3.0 ml	2.5 ml
10% APS	100 μ l		
Temed	10 μ l		

3. Add APS to start the polymerization. Use large pipette tip to pour from one side of the plate. Carefully turn around and pour other gel.

4. Layer butanol on top of gel to get rid of bubbles and to set evenly.

5. Prepare stacking Gel:

	15%
H ₂ O	8.3 ml
2 M Tris pH 6.8	625 μ l
10% SDS	100 μ l
40% acrylamide	1 ml
10% APS	100 μ l

Temed	20 μ l
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6. Add temed, pour quickly and insert comb.
7. Place gel in running tank and add **1 x running buffer** to both inner and outer chambers. Wash wells out carefully. Remove bubbles from under the gel.
8. Load samples. 10 well combs hold 27 μ l. 15 well combs hold 16 μ l.
9. Run at 200v for 30' to 45'.

5 x Running Buffer (600ml)

Tris base	9 g
glycine	43.2 g
SDS	3 g