

BLATTER PROTEIN GEL FORMULA

Stock solutions:

- 30% (w/v) Acrylamide
- 1% (w/v) Bis-acrylamide
- 1M Tris, pH 8.9
- 1M Tris, pH 6.8
- 20% (w/v) SDS

The above solutions should be filtered (e.g., 0.45 μ m nitrocellulose).

10 x Reservoir Buffer

	<u>1 P</u>	<u>2 P</u>	<u>4 P</u>
Tris base	30 g	60 g	120 g
Glycine	144 g	288 g	576 g

Dilute 1:10 with dH₂O and add SDS to 0.1% (5ml of 20% SDS per litre of buffer).

Sample Buffer

	<u>for 50ml</u>
0.1M dithiothreitol	0.077 g
2% SDS	1 g or 5ml of 20%
0.08M Tris pH6.8	4 ml of 1M
15% Glycerol	7.5ml
Bromophenol blue is added until it "looks right"	

Running Gel

% Acrylamide	5%	7.5%	10%	12.5%	15%
% Bis-acrylamide	0.26%	0.195%	0.13%	0.103%	0.086%

Amount

(40 ml per gel)

30% Acrylamide	6.67ml	10.0ml	13.40ml	16.70ml	20.0ml
1% Bis	10.40	7.73	5.20	4.20	3.50
1M Tris pH 8.9	14.90	14.90	14.90	14.90	14.90
20% SDS	0.20	0.20	0.20	0.20	0.20
H ₂ O	7.73	7.10	6.20	3.90	1.30
TEMED	-----20μl-----				
10% APS	-----200μl-----				

5% Stacking Gel

Use 10ml for each gel

	<u>10ml</u>	<u>20ml</u>	<u>30ml</u>	<u>40ml</u>
30% Acrylamide	1.67ml	3.34ml	5.00ml	6.67ml
1% Bis	1.30	2.60	3.90	5.20
1M Tris pH6.8	1.25	2.50	3.75	5.00
20% SDS	0.05	0.10	0.15	0.20
H ₂ O	5.70	11.40	17.10	21.80
TEMED	10μl	20μl	30μl	40μl
10% APS	100μl	200μl	300μl	400*1

Buffer system is from Laemmli (1970) Nature 227: 680-685.

Acrylamide: Bis ratios are from Blattler et al., (1972) J. Chromat. 64: 147-155.