Tricine-SDS-PAGE

(ref Schagger, H., and von Jagow, G., Analytical Biochem. 1987, 166, 368-379)

Roztoky:

3x GB - 3x Gel Buffer (3M Tris HCl, 0.3% SDS, pH 8.45)

18.16 g Tris
10mL 5N HCl (86.2 mL 37% HCl + 113.8 mL H_2O), or 4.31mL 37%
0.75 mL 20% SDS
ad 50 mL H_2O

Cathode buffer (100 mM Tris, 100 mM Tricine, 0.1 % SDS, pH 8.25)

12.11 g Tris
17.92 g Tricine
5 mL 20 % SDS or 1 g SDS
ad 1000 mL H_2O

Anode buffer (100 mM Tris-HCl, pH 8.9)

12.11 g Tris
3 mL N HCl
ad 1000 mL H_2O

AB - Acrylamide/bisacrylamide mix (48% acrylamide, 1.5% bisacrylamide (99.5T, 3C))

24.0 g acrylamide
0.75 g bisacrylamide
ad 50 mL H_2O

3xSLB - sample lysis buffer (6% ME, 12% SDS, 150 mM Tris-HCl, 30% glycerol)

3 mL 0.5 M Tris-HCl, pH 7.0
0.6 mL mercaptoethanol
1.2 g SDS
3 mL glycerol
ad 10 mL H_2O (eventually add 5 mg Coomassie Brilliant Blue)

2D-LB - 2nd dimension lysis buffer (1% SDS, 1 % mercaptoethanol)

Fixing solution (50% metanol, 10% acetic acid)

Staining solution (0.025% Serva Blue G, 10 % acetic acid)

Destaining solution (10% acetic acid)

	Stacking 4%		Separating 10%		Separating 16%	
	2.5 mL	5 mL	5 mL	10 mL	5 mL	10 mL
3xGB	0.6 mL	1.2 mL	1.65 mL	3.3 mL	1.65 mL	3.3 mL
AB	0.2 mL	0.4 mL	1 mL	2 mL	1.65 mL	3.3 mL
water	1.68 mL	3.36 mL	1.82 mL	3.64 mL	1.18 mL	2.36 mL
glycerol	0	0	0.5 mL	1 mL	0.5 mL	1 mL
AP	20 uL	40 uL	25 uL	50 uL	15 uL	30 uL
TEMED	2 uL	4 uL	3 uL	6 uL	2 uL	4 uL