Tricine—Sodium Dodecyl Sulfate—Polyacrylamide Gel Electrophoresis for the Separation of Proteins 1 to 100 kDa

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- Tricine, as the trailing ion, allows resolution of smaller proteins at lower acrylamide concentrations. Great resolution for proteins between 5 and 20 kDa, and those above 30 kDa are already destacked within the sample gel.

STOCK SOLUTIONS FOR SDS-PAGE

Buffer	Tris (M)	Tricine (M)	pH	SDS (%)
Anode buffer	0.2	—	8.9[a]	—
Cathode buffer	0.1	0.1	8.25[b]	0.1
Gel buffer	3.0	—	8.45[a]	0.3

[a] Adjusted with HCl

[b] No correction of the pH, which is around 8.25

REMEMBER: SDS is harmful to pH meter electrodes. Add after pH-ing.

Acrylamide-bisacrylamide mixture	Percentage acrylamide (w/v)	Percentage bisacrylamide (w/v)
49.5% T, 3% C	48	1.5
49.5%, 6%	46.5	3.0

T denotes the total percentage concentration of both monomers (acrylamide and bisacrylamide)
C denotes the percentage concentration of the crosslinker relative to the total concentration T

COMPOSITION OF SEPARATING, "SPACER" AND STACKING GELS

			Separating gels			
	Stacking gel 4% T, 3% C	"Spacer" gel 10% T, 3% C	10% T, 3% C	16.5% T, 3% C	16.5% T, 6% C	16.5% T, 6% C w/ 6 M Urea
49.5% T, 3% C solution	1 ml	6.1 ml	6.1 ml	10 ml	—	—
49.5% T, 6% C solution	—	—	—	—	10 ml	10 ml
Gel buffer	3.1 ml	10 ml	10 ml	10 ml	10 ml	10 ml
Glycerol	—	—	4 g	4 g	4 g	—
Urea	—	—	—	—	—	10.8 g
Add water to final volume of	12.5 ml	30 ml	30 ml	30 ml	30 ml	30 ml

- 10% T, 3% C gel used as a uniform separating gel, only overlaid by 4% T, 3% C stacking gel (2 cm). Polymerized by addition of 150 µl of 10% ammonium persulfate and 15 µl TEMED/30 ml.
- All other gels: small-pore (16.5% T) overlaid by a spacer gel (2-3 cm), that was again overlaid by the stacking gel (1-2 cm). Small-pore gel and spacer polymerized by adding 100 µl of 10% APS and 10 µl TEMED to 30 ml of each mixture. Stacking gel polymerized by 100 µl of 10% APS and 10 µl TEMED per 12.5 ml.
- May omit spacer if resolution of proteins below 5 kDa is of minor interest.
- Start electrophoresis at 30 V constant for ~ 1 hr, until the samples completely leave sample pocket. See table 3 on original paper for voltages and currents depending on gel type and size.
- Fixed: 50% methanol, 10% acetic acid for 30 mins (0.7mm-gels) or 60 mins (1.6 mm-gels)
- Stained: 0.025% Serva blue G in 10% acetic acid for 1-2 hr
- Background destaining: 10% acetic acid for 2 hr (renewing ~ every 30 mins)

For 12 gels:

	Separating	Spacer	Stacking
49.5% T, 3% C	15 ml	4.6 ml	2.0 ml
Gel buffer	15 ml	7.5 ml	6.2 ml
50% glycerol	9.45 ml	—	—
H_2O	5.55 ml	10.4 ml	16.8 ml
TEMED	20 µl	15 µl	10 µl
APS	200 µl	150 µl	100 µl

For 1 gel:

	Separating	Spacer	Stacking
49.5% T, 3% C	1.25 ml	0.380 ml	0.170 ml
Gel buffer	1.25 ml	0.630 ml	0.520 ml
50% glycerol	0.790 ml	—	—
H_2O	0.460	0.870 ml	1.4 ml
TEMED	1.6 µl	1.25 µl	5 µl*
APS	16.6 µl	12.5 µl	10 µl*

*Probably too much

Stocks to make:

1 Liter 10X Anode Buffer
Tris 242.28 g
Use LOTS of concentrated HCl to lower pH to 8.9

1 Liter 10X Cathode Buffer
Tris 121.14 g
Tricine 179.2 g
SDS 10 g
pH should be around 8.25 without addition of acid or base

1 Liter of 1X Gel Buffer
Tris 363.42 g
Use LOTS of concentrated HCl to lower pH to 8.45
SDS 3 g

1 Liter of 1X Acrylamide-Bisacrylamide "49.5 T, 3% C"
WEAR GLOVES, MASK, COAT, AND GOGGLES!!!
Acrylamide 480 g
Bisacrylamide 15 g
Wrap in aluminum foil and store in 4°C

2X Tricine sample buffer (Current Protocols in Molecular Biology)
2 ml 4X Tris-HCl, pH 6.8 (0.5 M) [final 0.1 M]
2.4 ml (3.0 g) glycerol [24% final]
0.8 g SDS [8% final]
0.31 g DTT (0.2 M final)
2 mg Coomassie blue G-250 (0.02% final)
Add H_2O to 10 ml and mix