

Tricine Gels
(<~20kDa)

Procedure:

1. Assemble gel apparatus
2. Prepare gel solution for the resolving portion of the gel
(e.g., Two gels, 10% Acrylamide, 1.5mM spacers)

	Resolving gel (lower)	Stacking (upper)
(a) dH ₂ O	14ml	12.4ml
(b) 80% glycerol	6ml	-
(c) 3X gel buffer	20ml	5ml
(d) 30% acrylamide	20ml	2.6ml
(e) APS	dab (___10%)	dab (___10%)
(f) Temed	30μL	20μL
(g) Syringe in gel solution between glass plates		

(hint: use 18g11/2 needle for 1.5mM spacer thickness gels; 22g11/2 for 0.75mM spacer thickness)

- (h) Cover gel with a small aliquot of isopropanol, let polymerize (~15 min +)
- (i) Drain or wick off isopropanol from resolving gel
- (j) Insert comb into gel assembly apparatus
- (k) Prepare gel solution for the stacking portion of the gel
- (l) Syringe gel solution in between plates.
- (m) Let polymerize ~40min +
- (n) Remove comb
- (o) Wash wells w/ upper buffer
- (p) Add 2-ME to appropriate volume of 3X SAMPLE buffer
- (q) Add Appropriate vol. 3X sample buffer to sample
- (r) Incubate 10-20min (or more) at ~ 68°C
- (s) Assemble upper chamber onto gel
- (t) Add 1X lower buffer and 1X upper buffer to their respective chambers
- (u) Run ~20mAmp for 15 – 20h (can run up to 60mAmp but don't let it run off)

Solutions, buffers and stuff:

1. 3X gel buffer
 - (a) Tris base 181.5g
 - (b) HCl to pH 8.45
 - (c) SDS 1.5g
 - (d) dH₂O to 500ml
 - (e) f

2. 10X lower reservoir buffer
 - (a) Tris base 121g
 - (b) 12N HCl to pH 8.9
 - (c) dH₂O to 500ml

3. 10X upper reservoir buffer
 - (a) Tris base 60.5g
 - (b) Tricine 89.6g
 - (c) SDS 5.0g
 - (d) dH₂O to 500ml

- (e) No need to pH
- 4. 3X SDS Sample buffer
 - (a) 80% Glycerol 1.5ml
 - (b) 20%SDS 1.5ml
 - (c) 10X Upper buf
 - (d) BB
 - (e) 1uL 2-ME per 20μL immediately before use
- 5. 30% Acrylamide (100ml)
 - (a) Acrylamide 29g
 - (b) Bis-acrylamide 1g
 - (c) dH₂O to 100ml
 - (d) Filter through 0.45μM filter
 - (e) Ion exchange resin (BIO-RAD AG 501-X8 (D)) to cover bottle bottom
 - (f) Store in brown bottle @ 4°C

Notes:

Acrylamide is a neurotoxin. It is advisable to work in a fume hood when weighing the stuff out. Once polymerized acrylamide is supposedly benign