

EARNSHAW LAB PROTEIN GEL PROTOCOL

LOWER GEL

RECIPE FOR 1.5 mm SPACERS. (FOR 0.75 mm MAKE HALF AS MUCH.)

	FINAL PERCENTAGE						
	7.5%	10%	12.5%	13%	13.5%	15%	16%
LOWER BUFFER	10	10	10	10	10	10	10 ml
ACRYLAMIDE	10.8	13.4	16.6	17.3	18	20	20.6 ml
20% SDS	0.2	0.2	0.2	0.2	0.2	0.2	0.2 ml
H ₂ O	18.8	16.2	13	12.3	11.6	9.6	9.0 ml
100 mM EDTA	0.4	0.4	0.4	0.4	0.4	0.4	0.4 ml
10% AMPS	0.4	0.4	0.4	0.4	0.4	0.4	0.4 ml
TEMED	40µl	40µl	40µl	40µl	40µl	40µl	40µl

layer H₂O-saturated isobutanol over the top

STACKING GEL

UREA TO 8 M 9.6 gm/(20 ml)

UPPER BUFFER 5 ml

ACRYLAMIDE 2.67 ml

20% SDS 0.1 ml

100 mM EDTA 0.2 ml

H₂O TO 20 ml

Heat in 37° bath - swirl to dissolve urea **THEN ADD**

AMPS 0.2 ml

TEMED 20 ml

SDS-POLYACRYLAMIDE GEL PROTOCOL

Let polymerize ≤ 15 minutes (10 min. is probably sufficient)

SDS-POLYACRYLAMIDE GEL PROTOCOL

PROTEIN GEL STOCK SOLUTIONS

10x RUNNING BUFFER (For use dilute 1:10 and add SDS to 0.1%)

trizma base	121.2 gm	(25 mM final)
glycine (Aldrich)	576.8	(192 mM final)

H ₂ O to 4 liters		

LOWER GEL BUFFER (1.5 M Tris:HCl pH 8.8)

trizma base	182 gm
pH adjust to 8.8 with conc. HCl	

H ₂ O to 1 liter - 0.2 μM filter	

UPPER GEL BUFFER (0.5 M Tris:HCl pH 6.8)

trizma base	61 gm
pH adjust to 6.8 with conc. HCl	

H ₂ O to 1 liter - 0.2 μM filter	

ACRYLAMIDE STOCK (30% Acrylamide:0.8% Bis-acrylamide)

acrylamide	300 gm
bis-acrylamide	8.0 gm
H ₂ O to 1 liter - 0.2 μM filter	

store in brown bottle at 4°

3x SAMPLE BUFFER (Kistler/ Gerace combination)

	for 100 ml 3x	final conc. in sample
Tris:HCl pH 6.8	30 ml of 0.5 M	50 mM Tris:HCl pH 6.8
Sucrose	45 gm best grade	15% Sucrose
K-EDTA	6 ml of 0.1 M pH 7.4	2 mM EDTA
SDS	30 ml 20% + 3 gm solid	3% SDS
Bromophenol Blue	0.1 gm	

make to 100 ml - aliquot into 3 ml aliquots & store @ -20°

for 1x -	300 μl 3x SB	or	300 μl 3x SB
	600 μl H ₂ O		510 μl H ₂ O
	20 μl 1M DTT		90 μl β-mercaptoEtOH

(1 M DTT - 1.542 gm in 100 ml H₂O stored @ - 20°)

STAINING AND DESTAINING GELS

STAINING

0.5% Coomassie Blue R in MeOH	50 ml
35% MeOH, 14% Acetic Acid	200 ml

FAST DESTAIN

35% MeOH
10% Acetic Acid

SLOW DESTAIN

10% MeOH
7% Acetic Acid

use 2 kimwipes to soak up stain - change as necessary