GENERAL REAGENTS- Protein Work

IMMUNOPRECIPITATIONS:

BSA (bovine serum albumin)
Lysis Buffer:
50 mM Tris, pH 7.4
150 mM NaCl
1% Triton X-100
100 mM EDTA, pH 8.0
1:100 of 0.1 M PMSF in 100% EtOH
1:1000 of 5 mg/mL leupeptin in dH_2O
1:1000 of 1.4 mg/mL pepstatinA in MeOH

SDS-PAGE GELS:

SDS Sample Buffer

Lower Gel Buffer:
36.4 g Tris in 100 mL dH_2O
pH to 8.8
add 0.8 g SDS

Upper Gel Buffer:
6.05 g Tris Base in 100 mL dH_2O
pH to 6.8
Add 0.4 g SDS

10X Laemelli Running Buffer:
For 1L:
30.3 g Tris Base

144.1 g Glycine

10g SDS

pH should be 8.3

# gels→	**1**	**2**	**3**	**4**
5%				
Acrylamide	0.62	1.25	1.87	2.5
Lower Gel Buffer	1.25	2.50	3.75	5.00
dH_2O	3.13	6.26	9.4	12.25
TOTAL	5	10	15	20
8%				
Acrylamide	1	2	3	4
Lower Gel Buffer	1.25	2.50	3.75	5.00
dH_2O	2.75	5.50	8.25	11.00
TOTAL	5	10	15	20
10%				
Acrylamide	1.25	2.50	3.75	5.00
Lower Gel Buffer	1.25	2.50	3.75	5.00
dH_2O	2.5	5.0	7.5	10
TOTAL	5	10	15	20
12%				
Acrylamide	1.5	3	4.5	6
Lower Gel Buffer	1.25	2.50	3.75	5.00
dH_2O	2.25	4.5	6.75	9
TOTAL	5	10	15	20
Upper Gel				
Acrylamide	0.28	0.56	0.85	1.13
Upper Gel Buffer	0.63	1.25	1.88	2.5
dH_2O	1.6	3.2	4.8	6.4
TOTAL	2.5	5.0	7.5	10.0

**This is for 0.75 mm*	*gels.*	*Double*	*for 1.5mm*	*gels.*
**For 5 mL gel solution*	*add*	*10 uL*	*TEMED*	
	and	*20 uL*	*10% APS*	

WESTERN BLOTTING:

Transfer Buffer: for 1L

5.8 g Tris Base

2.9 g glycine

0.37 g SDS

---Make to 800 mL with dH_2O, then add 200 mL MeOH---

Blocking Solution: for 1L

10 g powdered nonfat milk (1%)

500 uL Tween 20 (0.05%)

Make to 1L with 1X PBS

Store at 4°C for no more than 1 week.

Horseradish Peroxidase Developer:

10 mL MeOH

30 mg 4-chloro-1-naphthol

Make to 50 mL with 1X PBS.

Add 10 uL H_2O_2 and mix.

Good for less than 1 hour.

Alkaline Phosphatase Developer (APC)*:*

APC Solution

100 mM Tris-HCl

100 mM NaCl

5 mM $MgCl_2$

pH to 9.5

SILVER STAINING:

Fixer:
20 mL dH_2O
25 mL methanol
5 mL acetic acid

Developer:
1.5 g Na_2CO_3
125 uL formaldehyde
250 mL dH_2O

PROTEIN SAMPLES:

10X Wash Buffer (for protein samples):
0.1 mM MES pH 6.8
2.5 mM EGTA
1 mM $MgCl_2$

Lysis Buffer: (for 20 mL)
2 mL 10X Wash Buffer (for protein samples)
10 uL of 20% Triton X-100
2 uL of 5 mg/mL Leupeptin
20 uL of 0.1 M PMSF (in 100% EtOH-stored at 4°C)
2 uL of 1.4 mg/mL pepstatinA

2X Sample Buffer: For 10 mL

100 mM Tris Base	0.12 g
200 mM DTT	0.31 g
4% SDS	0.40 g
0.2% Bromophenol Blue	tip of small spatula
20% glycerol	2 mL of 100%

COOMASSIE STAINING:

Staining Solution:
450 mL MeOH + 2.5 g Coomassie Brilliant Blue R.
Swirl until dissolved.
Add 100 mL acetic acid and 450 mL water.

Destaining Solution:

450 mL MeOH + 100 mL acetic acid + 450 mL of water. (everything but the R250)