

# Western Blot Buffers

## 10x/20x (run/transfer) Tris Glycine Buffer

30.3g Tris Base

114.2g Glycine

Add to 1L with ddH<sub>2</sub>O

to make 1x SDS running buffer, make 1L of 1X (100mL of Tris/Gly buffer stock) then add 10mL of 10% SDS – makes 0.1% SDS

to make 1L of 1x transfer, add:

50mL of Tris/Gly buffer stock

100mL (10%) methanol

850mL water

2x SDS sample buffer:

20mL glycerol

32mL 10% SDS

12.5mL Tris pH6.8

water to **100 mL**

+ bromophenol blue

## 10 X Phosphate Buffered Saline (PBS)

1. Dissolve 80g of NaCl, 2.0g of KCl, 14.4g of Na<sub>2</sub>PO<sub>4</sub>, and 2.4g of KH<sub>2</sub>PO<sub>4</sub> in 800ml distilled H<sub>2</sub>O.

2. Adjust pH to 7.4 with HCl.

3. Adjust volume to 1L with additional distilled H<sub>2</sub>O.

1X PBS Wash Buffer: (1X PBS + 0.05% TWEEN-20 detergent)

100mL 10x PBS

2.5 mL of 20% TWEEN-20

~900mL ddH<sub>2</sub>O

\*\*\* use for blot washing and antibody dilution

Also make:

1M Tris-HCl pH 6.8:

15.76g in 90mL ddH<sub>2</sub>O, adjust pH to 6.8, q.s. ddH<sub>2</sub>O to 100mL

1 M Tris-HCl pH 8.8:

15.76g in 90mL ddH<sub>2</sub>O, adjust pH to 8.8, q.s. ddH<sub>2</sub>O to 100mL

10% SDS solution: 10g SDS in 100mL ddH<sub>2</sub>O, stir on stirplate overnight.

30% polyacrylamide solution: Acrylamide/Bis-acrylamide (30%/0.8% w/v), 30g acrylamide, 0.8g bis-acrylamide in 100mL ddH<sub>2</sub>O

	<u>Final Resolving Gel Percentage</u>			
	<u>9%</u>	<u>10%</u>	<u>12%</u>	<u>15%</u>
<u>Stock Solution</u>				
1M Tris pH 8.8	3.75	3.75	3.75	3.75
10% SDS	100uL	100uL	100uL	100uL
30% Acrylamide	3	3.3	4	5
H <sub>2</sub> O	3.05	2.7	2.05	1.05
10% Ammonium persulfate	100 µL	100 µL	100 µL	100 µL
TEMED	5 µL	4 µL	4 µL	4 µL
10mL total gel mix volume				

	<u>Vol in mL</u>
	<u>Stock Solution</u>
1M Tris pH 6.8	0.63
10% SDS	50uL
30% Acrylamide	0.83
H <sub>2</sub> O	3.4
10% Ammonium persulfate	50 µL
TEMED	5 µL
5mL total gel mix volume	