

## ELISA REAGENTS

1. Carbonate coating buffer, pH 9.6. Do not have to filter or autoclave.

Na <sub>2</sub> CO <sub>3</sub>	7.95 g	or	34.98 g
NaHCO <sub>3</sub>	14.65 g		64.46 g
NaN <sub>3</sub> (optional)	<u>1.00 g</u>		<u>4.40 g</u>
Distilled water to:	500 ml		2,200 ml

2. Phosphate Buffered Saline (PBS), pH 7.4 (make 10x). Use 1/10 for final concentration of 0.02M Phosphate + 0.15M NaCl. Can add 2gm/1L of NaN<sub>3</sub> to 10x solution to prevent bacterial growth.

NaH <sub>2</sub> PO <sub>4</sub> (anh)	9.04 g	0.0376M
Na <sub>2</sub> HPO <sub>4</sub> (anh)	46.00 g	0.1620M
NaCl	<u>175.00 g</u>	1.5000M
Distilled water to:	2,000 ml (=0.2M/L)	

- or -

NaH <sub>2</sub> PO <sub>4</sub> *12H <sub>2</sub> O	11.7 g
Na <sub>2</sub> HPO <sub>4</sub> *12H <sub>2</sub> O	116.0 g
NaCl	<u>87.5 g</u>
Distilled water to:	1,000 ml

3. PBS-Tween (washing solution)

10x PBS	400 ml	
2% NaN <sub>3</sub> (Azide)	40 ml	0.02 %
Tween 20	<u>2 ml</u>	0.05 %
Distilled water to:	4,000 ml	

4. PBS-Tween with 0.1% BSA (diluent)

10x PBS	100.0 ml	
2% NaN <sub>3</sub> (Azide)	10.0 ml	0.02 %
Tween 20	0.5 ml	0.05 %
BSA	<u>1.0 g</u>	0.10 %
Distilled water to:	1,000 ml	

## 5. PBS with 0.1% BSA (blocking solution)

10x PBS	100 ml	
2% NaN <sub>3</sub> (Azide)	10 ml	0.02 %
BSA	<u>1 g</u>	0.10 %
Distilled water to: 1,000 ml		

## 6. Substrate buffer, pH 8.6

NaHCO <sub>3</sub>	14.03 g	0.167 M	
Na <sub>2</sub> CO <sub>3</sub> (anh)	1.30 g	0.012 M	***Add slowly to sol'n. w/stirring
MgCl <sub>2</sub> *6H <sub>2</sub> O	0.20 g	0.001 M	
NaN <sub>3</sub> (Azide)	<u>2.00 g</u>	0.02 %	
Distilled water to: 1,000 ml			

## 7. Substrate solution, 1 mg/ml Store at -20°C in 10 ml aliquots. Keep protected from light.

PNPP	20 mg tablet
Substrate buffer	20 ml