



**APPENDIX E**

สำนักหอสมุด

## Reagents for Western blot analysis

### 1. Transfer buffer (blotting buffer, pH 8.3)

To prepare 1,000 ml of this buffer, 4.03 g of Tris base and 14.4 g of glycine were dissolved in 800 ml of UDW. Subsequently, 200 ml of methanol was added to yield 20%. This solution was kept at 4°C until use.

### 2. Phosphate buffered saline (0.01 M PBS, pH 7.4)

Ten milliliters of 1.0 M PB, pH 7.4 and 50 ml of 3M NaCl were added with DW and the volume was brought up to 1,000 ml with DW.

### 3. Phosphate buffer (1/15 M PB, pH 7.6)

This solution was prepared by dissolving 0.06 g of Na<sub>2</sub>PO<sub>4</sub> and 0.473 g of Na<sub>2</sub>HPO<sub>4</sub> in 57.7 ml UDW. The pH was then adjusted to 7.6 with 1 N HCl.

### 4. Tris-HCl buffer (0.15 M Tris HCl, pH 9.6)

Tris-HCl (18.16 g) was dissolved in 1 liter of DW, pH was adjusted to 9.6 with HCl.

### 5. Washing buffer (0.05% Tween-20 in PBS, pH 7.4)

This solution was prepared by adding 0.5 ml of Tween-20 in 1 liter of 0.01 M PBS, pH 7.4.

### 6. Blocking buffer (3% BSA, 0.5% gelatin in PBS, pH 7.4)

Gelatin (0.5 g) was added in 100 ml of 0.01 M PBS, pH 7.4. The preparation was warmed up to 45-50°C to melt the gelatin and then cooled down to 25°C. BSA (3 g) was then added to the gelatin solution to completely dissolve.

**7. Diluent**

Gelatin (0.2 g) was dissolved to 100 ml of the 0.01 M PBS, pH 7.4. The preparation was warmed up to 45-50°C to melt the gelatin and cooled down to 25°C. BSA (0.2 g) was added and mixed to dissolve completely.

**8. Horseradish peroxidase (HRP) substrate solution**

This solution was prepared by dissolving 0.02 g of 2', 6' dichlorophenol indophenol (Sigma Chemical Co.) in 10 ml of 1/15 M PB, pH 7.6. Three microliters of 30% H<sub>2</sub>O<sub>2</sub> was added before use.

**9. Alkaline phosphatase substrate solution (BCIP/NBT)**

Commercial BCIP/NBT substrate solution was purchased from KPL, USA. To prepare this solution, one part of concentrate substrate was diluted with three part of diluent (0.15 M Tris-HCl, pH 9.6). This substrate should be prepared freshly and protected from the light.