# APPENDIX H

**Reagents for Western blot analysis**

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

[**25mM Tris, 192 mM glycine and 20% (v/v) methanol**]

To prepared 4,000 ml of this buffer, 12.12 g of Tris base (hydroxymethyl aminomethane) and 57.60 g of glycine was dissolved in 3,200 ml of UDW. Subsequently, 800 ml of methanol was added to yield 20% (v/v).

**2. Tris buffer (0.15 M Tris-HCl, pH 9.6)**

The buffer was prepared by dissolving 18.15 g of Tris-base in 500 ml of deionized distilled water. The pH of this solution was adjusted to 9.6 with 1 N HCl. The final volume was brought up to 1,000 ml with distilled water.

**3. Phosphate buffered saline (0.01 M PBS, pH 7.4)**

The solution was prepared by dissolving 1.22 g of anhydrous $Na_2HPO_4$, 0.17 g of anhydrous $NaH_2PO_4$ and 8.77 g of NaCl in 1 liter of DW. The pH of this solution was adjusted to 7.4 with 1 N HCl.

**4. Washing solution (PBST)**

The PBST was prepared by mixing Tween-20 in PBS, pH 7.4 to a 0.05 % concentration.

**5. Blocking solution**

The solution was prepared by dissolving 5 g of skim milk (Fraction V, Sigma Chemical Co., St Louis, Minnesota, USA) in 100 ml of 0.01 M PBS, pH 7.4.

**6. Detection reagents**

The anti-E-Tag MAb (GE Healthcare), goat anti-mouse immunoglobulins alkaline phosphatase conjugate (Southern), and goat anti-mouse immunoglobulins hydrogenperoxidase conjugate (Southern), were prepared by diluting in PBST to the desired dilution.

**7. Substrate solution**

Commercially avialable BCIP/NBT substrate solution for alkaline phosphatase in concentrate form was purchased from Kirkegaard & Perry Laboratory (KPL), USA. To prepare the working solution, one part of concentrate substrate was diluted with three part of 0.15 M Tris-HCl, pH 9.6. This working substrate solution was freshly prepared and always protected from the light.