APPENDIX D

Reagent for Western blot analysis

1. Transfer buffer (bloting 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 this base (hydroxymethyl aminomethane (Sigma chemocal Co., USA) and 57.60 g of glycine were dissolved in 3,200 ml of UDW. Subsequently, 800 ml of methanol was added to yeild 20% (v/v).

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

This 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 M HCl.

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

The buffer was prepared by dissolving 0.06 g of NaH_2PO_4 and 0.47 g of Na_2HPO_4 in 57.7 ml of UDW. The pH of this solution was adjusted to 7.6 with 1 N HCl.

4. Washing buffer

This solution was prepared by adding 0.5 ml of Tween-20 in one liter of 0.01 M PBS pH 7.4 and mixed well.

5. Blocking solution (3% BSA, 0.5% gelatin, in PBS, pH 7.4)

The solution was prepared by dissolving 3 g of bovine serum albumin (BSA, Sigma chemical Co., USA) and 0.5 g of gelatin (Sigma) in 100 ml of 0.01 M PBS, pH 7.4.

6. Conjugate solution

This solution was prepared by diluting rabbit anti-mouse immunoglulins-peroxidase conjugate (DAKO, Denmark) with diluent solution to make the desired diltuion.

7. Diluent solution

The solution was prepared by dissolving 0.2 g of BSA and 0.2 g of gelatin in 100 ml of 0.01 M PBS, pH 7.4.

8. Substrate solution

The solution was freshy prepared by dissolving 0.02 g of 2,6-dichloro-phenol indophenol (BSA, Sigma) in 10 ml of 1/15 M PB, pH 7.6. Ten µl of 30% H_2O_2 was added immediately before use.