



APPENDIX D

สำนักหอสมุด

Reagents for indirect ELISA

1. Coating buffer (Carbonate-bicarbonate buffer, pH 9.6)

The buffer was prepared by dissolving 1.26 g of NaHCO_3 in 300 ml of DW, then the pH was adjusted to 9.6 with 0.05 Na_2CO_3 (0.53 g in 100 ml of DW).

2. 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.

3. Washing solution (PBST)

Washing solution (PBST) was prepared by mixing Tween-20 in PBS, pH 7.4 to a 0.05 % concentration.

4. Blocking solution

The solution was prepared by dissolving 1 g of BSA (Fraction V, Sigma Chemical Co., St Louis, Minnesota, USA) in 100 ml of 0.01 M PBS, pH 7.4.

5. Diluent solution

The diluent 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.

6. Substrate buffer (0.1 M citrate buffer, pH 4.5)

The buffer was prepared by dissolving 14.7 g of trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot \text{H}_2\text{O}$) in DW. The volume was made up to 500 ml after the pH was adjusted to 4.5 with 1M HCl.

7. Substrate solution

The substrate solution consisted of 0.05% 1, 4- *p*-phenylenediamine-dihydrochloride (PPD) (Sigma Chemical Co.) in citrate buffer, pH 4.5 and 0.01% of 30% H_2O_2 . This solution was prepared freshly before use and always protected from light.

8. Stop solution (1N NaOH)

The solution was prepared by dissolving 20 g of NaOH in 500 ml of UDW.