



APPENDIX C

สำนักหอสมุด

Reagents for indirect ELISA and phage ELISA

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

This buffer contained 2.93 g of NaHCO₃ (Merck, Germany) and 0.53 g of Na₂CO₃ (Merck) in 900 ml of DW. The pH was adjusted to 9.6 with NaOH and the volume was made up to 1 liter.

2. Phosphate buffer (1 M PB, pH 7.4)

Solution A

Na ₂ HPO ₄ ·2H ₂ O (Merck, Germany)	177.90	g
DW to	1,000	ml

Solution B

NaH ₂ PO ₄ ·2H ₂ O (Merck, Germany)	156.01	g
DW to	1,000	ml

The solution B was added to solution A for adjusting the pH to 7.4.

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

Ten milliliters of the 1.0 M PB, pH 7.4 and 50 ml of 3M NaCl (Univar, USA) were added in 900 ml of DW. DW was added to make 1000 ml of the volume.

4. Washing solution (PBS-T)

The washing solution (PBS-T) was prepared by mixing Tween-20 (Sigma Chemical Co.) in 0.01 M PBS, pH 7.4 to a 0.05% final concentration.

5. Blocking solution (1% BSA) (for indirect ELISA)

One gram of BSA (Nacalai tesque, Japan) was completely dissolved in 100 ml of 0.01 M PBS, pH 7.4.

6. Blocking solution (2% BSA) (for phage ELISA)

BSA (2 g) was completely dissolved in 100 ml of 0.01 M PBS, pH 7.4.

7. Diluent (0.2% BSA, 0.2% gelatin)

The diluent solution consisted of 0.2 g of BSA and 0.2 g of gelatin (Sigma Chemical Co.) in 100 ml of warm PBS, pH 7.4. Then the solution was cooled down to 25°C before adding BSA to dissolve.

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

Trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot \text{H}_2\text{O}$) (Merck) (14.7) g was dissolved in 400 ml of DW. The pH of the solution was then adjusted to 4.5 with concentrate HCl (Merck) and the final volume was made up to 500 ml with DW.

9. Substrate solution (for indirect ELISA only)

The substrate solution consisted of 0.05% (0.005 g) of 1,4-*p*-phenylenediamine-dihydrochloride (PPD) (Sigma Chemical Co.) in citrate buffer pH 4.5 (10 ml), and 0.01% (10 μl) of 30% H_2O_2 (Merck). This solution was freshly prepared before use and always protected from light.

10. Stop solution (1 N NaOH)

NaOH (Merck) (20 g) was dissolved in 500 ml of distilled water.