



APPENDIX G

สำนักหอสมุด

Reagents for Western blot analysis and 2-D immunoblotting

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 buffered saline (0.01 M TBS, pH 7.4)

Tris-base 1.21 g and 8.77 g of sodium chloride was dissolved in 500 ml of deionized distilled water. The pH was adjusted to 7.4 with HCl then the final volume was brought up to 1,000 ml.

3. 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.

4. Washing buffer (0.05% Tween-20 in TBS, pH 7.6; TBST)

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

5. Blocking solution (3% BSA, in TBS, pH 7.6)

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

6. Diluent solution (0.2% BSA in TBS, pH 7.6)

The solution was prepared by dissolving 0.2 g of bovine serum albumin (BSA, Sigma Chemical Co.) in 100 ml of 0.01 M TBS, pH 7.6

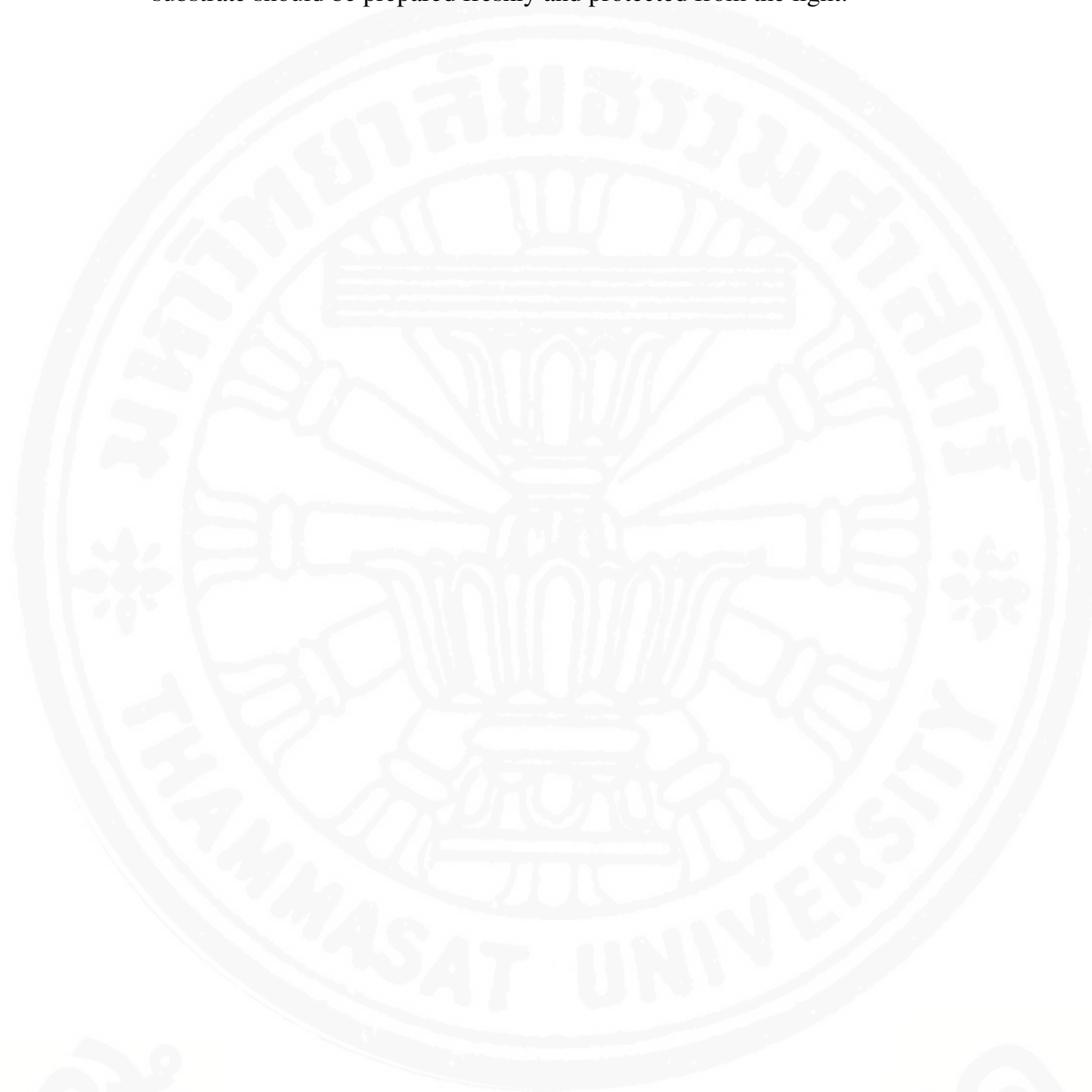
7. Conjugate solution

This solution was prepared by diluting goat anti-human immunoglobulins G alkaline phosphatase conjugate (Southern) with diluent solution to make the desired dilution.

8 Substrate solution

Commercial BCIP/NBT substrate solution was purchased from Kirkegaard & Perry Laboratory (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.



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