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APPENDICES

APPENDIX I

CHEMICAL AGENTS AND INSTRUMENTS

A. Chemical substances

Acrylamide (Bio-Rad, CA, USA)

Agarose (Sigma, MO, USA)

Ammonium persulphate (E. Merck, Darmstadt. W. Germany)

Boric acid H_3BO_3 (Bio-Rad, CA, USA)

Bromphenol blue (E. Merck, Darmstadt. W. Germany)

Cancium chloride anhydrous $CaCl_2$ (E. Merck, Darmstadt. W. Germany)

Chroloform (E. Merck, Darmstadt. W. Germany)

Citric acid $C_6H_8O_7.H_2O$ (E. Merck, Darmstadt. W. Germany)

Coomassie Brilliant Blue dye R-250 (Bio-Rad, CA, USA)

o-diphenylenediamine (Sigma, MO, USA)

Ethylenediamine tetra-acetic acid EDTA (disodium salt) (BDH, Poole, England)

Ethyleneglycol-bis-(β-aminoethylether)N,N,N',N'-tetra-acetic acid EGTA (Sigma, MO, USA)

Ethanol CH_3OH (E. Merck, Darmstadt. W. Germany)

Glacial acetic acid (E. Merck, Darmstadt. W. Germany)

Glycerol (Carlo Erba, Milano, Italy)

Glycine $H_2NCH_2.COOH$ (E. Merck, Darmstadt. W. Germany)

Hydrochloric acid HCl (E. Merck, Darmstadt. W. Germany)

Isoamyl alcohol (E. Merck, Darmstadt. W. Germany)

2-mercaptoethanol (E. Merck, Darmstadt. W. Germany)

Methanol (Carlo Erba, Milano, Italy)

Magnesium chloride anhydrous $MgCl_2$ (E. Merck, Darmstadt. W. Germany)

Phenol C_6H_5OH (E. Merck, Darmstadt. W. Germany)

Phenylmethylsulfonyl fluoride (PMSF) (Sigma, MO, USA)

Poly-L-lysine (Sigma, MO, USA)

Potassium chloride KCl (E. Merck, Darmstadt. W. Germany)

Saccharose $C_{12}H_{22}O_{11}$ (Sigma, MO, USA)

Sephacryl S-300 (Amersham Biosciences AB, Uppsala, Sweden)

Sodium chloride NaCl (Carlo Erba, Milano, Italy)

Sodium hydrogen carbonate $NaHCO_3$ (E. Merck, Darmstadt. W. Germany)

Sodium carbonate anhydrous Na_2CO_3 (E. Merck, Darmstadt. W. Germany)

Sodium azide NaN_3 (Carlo Erba, Milano, Italy)

Sodium dihydrogen phosphate NaH_2PO_4 (E. Merck, Darmstadt. W. Germany)

Di-sodium hydrogen phosphate Na_2HPO_4 (E. Merck, Darmstadt. W. Germany)

Sodium hydroxide NaOH (E. Merck, Darmstadt. W. Germany)

Sodium dodecyl sulphate (SDS) (Bio-Rad, CA, USA)

Spermine (Sigma, MO, USA)

Spermidine (Sigma, MO, USA)

Sulfuric acid H_2SO_4 (J.T.Baker, NJ, USA)

TEMED (Bio-Rad, CA, USA)

Tris-base (Sigma, MO, USA)

Triton X-100 (Sigma, MO, USA)

Tween 20 (Sigma, MO, USA)

B. Antiserum and serum

Rabbit peroxidase-conjugated antihuman immunoglobulin G (DAKO, Glastrup, Denmark)

Fetal calf serum (GIBCO BRL, Germany)

Anti-C3 (Behring AG, Marburg, Germany)

Anti-C4 (Behring AG, Marburg, Germany)

C. Enzyme

Micrococcal nuclease (Sigma, MO, USA)

D. Markers

Prestained protein marker, Broad Range (New England Biolabs, USA)

DNA marker, 100 base pairs (Promega co., WI, USA)

E. Cell culture

Human epithelial (HEp-2) cells (ANAFAST Diasorin, USA)

F. Glasswares

Beaker (pyrex, Corning, N.Y., USA)

Cylinder (Witeg, W. Germany)

Erlenmeyer flask (pyrex, Corning, N.Y., USA)

Glass tube (pyrex, Corning, N.Y., USA)

Microtiter plate (96 wells, flat bottom) (Nunc-immuno plate Maxisorp, Nunc, Denmark)

G. Instruments

Amicon PM-30 filters (Amicon, Lexington, MA)

Analytical balance (Precisa, Switzweland)

Automatic pipette (Gilson, Lyon, France)

Centrifuge, model 2K15 (Sigma, MO, USA)

Electrophoretic tank (Gelmate 2000, Japan)

ELISA reader, model 311.CO (Organon Teknika, Belgium)

ELISA washer (Washer 400 Organon Teknika, Belgium)

Fluorescence microsope, model C-35AD-4 (Olympus, Japan)

Fraction collector, LKB Superac (LKB-Produkter AB, Bromma, Sweden)

Mini-Protean II cells (Bio-Rad, CA, USA)

Mixer Voetex-Genie 2 (Scientfic industries, NY, USA)

Nephelometer (Behring AG, Marburg, Germany)

pH-meter, model 520A (Orion, MA, USA)

Power supply, model 2197 (LKB BROMMA, Sweden)

Spectrophotometer (Bio-Rad, CA, USA)

APPENDIX II

NUCLEOSOME PREPARATION

Washing buffer, pH 7.5

Washing buffer consists of 15 mM Tris buffer containing 15 mM NaCl, 60 mM KCl, 2mM EDTA, 0.5 mM EGTA, 0.15 mg/ml spermine, 0.5 mM spermidine, 0.34 M saccharose, 15 mM 2-mercaptoethanol, and 0.2 mM phenylmethylsulfonyl fluoride (PMSF).

1 M Tris-HCl, pH 6.8	15	ml
NaCl	0.88	g
KCl	4.48	g
EDTA	0.75	g
EGTA	0.19	g
Spermine	0.15	g
Spermidine	0.07	g
Saccharose	116.38	g
2-mercaptoethanol	0.52	ml
Phenylmethylsulfonyl fluoride (PMSF)	0.04	g

This solution was prepared by dissolving all of reagents in 800 ml of DW. The pH of this solution was adjusted to 7.5 with 1 N HCl and made up the volume to 1000 ml with DW. Stored the solution at 4 °C.

Lysis buffer

This lysis buffer contains 0.05% Triton X-100.

Triton X-100	0.5	ml
Washing buffer, pH 7.5	99.5	ml

Digestion buffer, pH 7.4

A digestion buffer consists of 50 mM Tris, 25 mM KCl, 4 mM $MgCl_2$, 1 mM $CaCl_2$, and 0.2 mM PMSF.

1 M Tris-HCl buffer, pH 6.8	50	ml
KCl	1.46	g
$MgCl_2$	0.34	g
$CaCl_2$	0.11	g
Phenylmethylsulfonyl fluoride (PMSF)	0.035	g

This solution was prepared by dissolving all of reagents in 800 ml of DW. The pH of this solution was adjusted to 7.4 with 1 N HCl and made up the volume to 1000 ml with DW. Stored the solution at 4 °C.

Extraction buffer, pH 7.5

An extraction buffer consists of 50 mM Tris, 0.25 mM EDTA, 0.02% NaN_3, and 0.2 mM PMSF.

1 M Tris-HCl buffer, pH 6.8	50	ml
EDTA	0.093	g
Phenylmethylsulfonyl fluoride (PMSF)	0.035	g
NaN_3	0.2	g

This solution was prepared by dissolving all of reagents in 800 ml of DW. The pH of this solution was adjusted to 7.5 with 1 N HCl and made up the volume to 1000 ml with DW. Stored the solution at 4 °C.

APPENDIX III

SODIUM DODECYL SULPHATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

Sample buffer

1 M Tris-HCl, pH 6.8	3.125	ml
SDS (10% solution)	2.30	ml
Glycerol	10	ml
0.1% Bromphenol blue	1	ml
β-mercaptoethanol	5	ml
and DW was added to	100	ml

This solution was stored at room temperature.

Acrylamide solution (30%)

To prepare this solution, 30 g of acrylamide and 0.8 g of N,N-methylacrylamide were dissolved in 70 ml of DW and then made up volume to 100 ml. The solution was filtered through a filter paper (Whatman No.1). this solution was stored at 4 °C in a brown bottle.

Tris-HCl (1.5 M, pH 8.8)

To prepare this solution, 18.2 g of Tris base (hydroxymethyl) aminomethane was dissolved in 50 ml of DW, then the pH was adjusted to 8.8 with 1 N HCl. The final volume was brought up to 100 ml with DW. Store this solution at room temperature.

Tris-HCl (1 M, pH 6.8)

To prepare this solution, 12.1 g of Tris base (hydroxymethyl) aminomethane was dissolved in 50 ml of DW, then the pH was adjusted to 6.8 with 1 N HCl. The final volume was brought up to 100 ml with DW. Store this solution at room temperature.

Sodium dodecyl sulphate (10% SDS)

This solution was prepared by dissolving 10 g of SDS in 100 ml of DW. Store this solution at room temperature.

Ammonium persulphate (10%)

This solution was prepared by dissolving 50 mg of ammonium persulphate in 0.5 ml of DW. Store at 4 °C.

Separating gel (15%)

Polyacrylamide separating gel (15%) was prepared by mixing the following ingredients together:

1.5 M Tris-HCl, pH 8.8	1.30 ml
30% acrylamide solution	2.00 ml
10% SDS solution	0.05 ml
DW	1.60 ml

The reagents were gently mixed. The polymerization was initiated by adding 0.05 ml of the 10% ammonium persulphate and 0.002 ml of TEMED. The gel was poured into the casting apparatus and was overlayed with DW. Allowed the mixture to polymerize at least 30 minutes at room temperature.

Stacking gel (5%)

The stacking gel (5%) was prepared by mixing the following reagents:

1M Tris-HCl, pH 6.8	0.25	ml
30% acrylamide solution	0.33	ml
10% SDS solution	0.02	ml
DW	1.40	ml

The reagents were gently mixed. The polymerization was initiated by adding 0.02 ml of the 10% ammonium persulphate and 0.002 ml of TEMED. The gel was poured on top of the separating gel which the DW had been removed, and allowed the mixture to polymerize at least 45 minutes at room temperature before use.

Electrode buffer (5 X Tris-glycine buffer, pH 8.3)

The buffer contained the following reagents:

Tris-base	15.1	g
Glycine	94.0	g
10% SDS solution	50	ml
and DW was added to	1000	ml

This mixture was stored at 4 °C.

Working electrode buffer (1 X)

This solution was prepared by diluting one part of the 5 x stock electrode buffer in 4 part of DW and adjusted pH to 8.3 with 1 N HCl. This solution can be reused 3 times. Store this solution at room temperature.

PROTEIN STAINING

Coomassie Brilliant Blue stain

Coomassie Brilliant Blue dye R-250 (0.25 g) was dissolved in 50 ml of absolute methanol before adding 10 ml of glacial acetic acid and 40 ml of DW. This solution was kept at room temperature.

Destaining solution

The solution was prepared by 100 ml of glacial acetic acid, 500 ml of methanol and added DW to make 1000 ml. The solution was kept at room temperature.

APPENDIX IV

AGAROSE GEL ELECTROPHORESIS

1.5% agarose gel

Agarose	0.4	g
Tris Borate-EDTA buffer, pH 8.3	20	ml

Tris Borate-EDTA buffer, pH 8.3

This solution consists of 44.5 mM Tris borate buffer, and 1 mM EDTA.

Tris-base	5.4	g
Boric acid	2.75	ml
0.5 M EDTA	2	ml

After dissolving all reagents in 800 ml of distilled water, this solution was adjusted pH to 8.3 by 1 N HCl and made up the volume to 1000 ml. Store the solution at room temperature.

APPENDIX V

INDIRECT ENZYME-LINKED IMMUNOSORBENT ASSAY
(Indirect ELISA)

Coating buffer (Carbonate-bicarbonate buffer, pH 9.6)

This buffer was prepared by dissolving 1.53 g of Na_2HCO_3 and 2.93 g of $NaHCO_3$ in 1 liter of DW and stored 4 °C, no longer than 2 weeks.

Phosphate buffered saline (0.01 M PBS, pH 7.4)

This solution was prepared by dissolving 1.2156 g of anhydrous Na_2HPO_4, 0.1700 g of anhydrous NaH_2PO_4 and 8.5160 g of NaCl in liter of DW. The pH of this solution was adjusted to 7.4 with 1 N HCl. Store this solution at room temperature.

Washing solution (PBS-0.05%T)

Washing solution (PBS-T) was prepared by mixing 0.5 ml of Tween 20 in PBS, pH 7.4.

Blocking solution

Blocking solution was prepared by adding 10 ml of fetal calf serum in 90 ml of PBS-0.05%T, pH 7.4.

Conjugate solution

The rabbit peroxidase-conjugated antihuman immunoglobulin IgG, store at 4 °C. Dilute stock solution in PBS-T-10% FCS to working dilution immediately before use.

Substrate buffer (0.1 M citrate buffer, pH 5.0)

The buffer was prepared by dissolving 4.735 g of Na_2HPO_4 and 3.65 g of citric acid in 450 ml of DW. After all ingredients were dissolved, the volume was made up to 500 ml. The pH was adjusted to 5.0 with 1 N HCl if necessary. This solution was kept at 4 °C in a brown bottle.

Substrate solution

The substrate solution consists of *o*-diphenylenediamine in a concentration of 0.4 mg/ml in the citrate buffer, pH 5.0 and 0.003% H_2O_2. This solution was prepared freshly before use and protected from light.

Stopping solution (1 N H_2SO_4)

The solution was prepared by adding H_2SO_4 13.8 ml in 300 ml of DW. Made up the volume with DW to 500 ml.

CURRICULUM VITAE

Miss Supannika Saisoong was born on February 22, 1978 in Nan, Thailand. She graduated with the Bachelor degree of Science in Microbiology from the Faculty of Science, Chulalongkorn University in 1998.