VIII. APPENDIX

Useful recipes

Culture Media

1. Brain heart infusion agar (BHA)

BHA agar (dehydrated)	47.0	g
Distilled water	1,000	ml

Melt, disperse in tubes and autoclave at 121°C 15 lbs for 5 min. Allow tubes cool in slant position.

2. Brain heart infusion broth (BHI)

BHI	37.0	g
Distilled water	1,000	ml

Disolve, disperse 50 ml in each 250 ml Erlenmeyer flask. Autoclave at 121°C 15 lbs for 15 min.

Reagents and Buffers

-For protein staining;

1. Amido black 10 B, 0.1%

Amido black 10 B	0.1	g
Acid-ethanol solution	100	ml

Acid-ethanol solution containing;

Absolute Ethanol	25	ml
Acetic acid	10	ml
Distilled water up to	100	ml

Stain nitrocellulose membrane for 1 min and destain with acid ethanol solution for 30-60 min.

2. Coomassie brilliant blue R-250, 0.1%

Coomassie brilliant blue R-250	0.1	g
Acid-methanol solution	100	ml

Acid-methanol containing ;

Methanol	40	ml
Acetic acid	10	ml
Distilled water up to	100	ml

Stain polyacrylamide gel for 30-60 min and destain with acid-methanol solution for 1-3 hr by several change volume.

-For immunoblot assay;

1. Blocking buffer

Non-fat dry milk	5.0	g
PBS, pH 7.2	100	ml

2. Chloronaphthol solution (chromogen stock solution)

4-Chloro-1-naphthol	0.3	g
Absolute ethanol	10	ml

Store at -20°C for at least 1 year.

3. Working chromogenic substrate solution containing ;

Stock chloronaphthol solution	0.1	ml
50 mM Tris-HCl, pH 7.6	10.0	ml

Remove the white precipitation by filtering through Whatman no.1 filter paper. Before using add 10 ul of 30% H_2O_2.

4. Phosphate buffer saline (PBS), pH 7.2

NaCl	8.0	g
KCL	0.20	g
Na_2HPO_4	1.15	g
KH_2PO_4	0.20	g
Distilled water	1,000	ml

Adjust pH to 7.2 with 1 N HCl.

5. PBS-Tween (PBS-T)

Phosphate buffer saline, pH 7.2	100	ml
Tween 20	0.05	ml

-<u>For enzyme inhibition</u>;

1. Ethylene diaminetetra acetic acid (EDTA), 500 mM pH 8.5

EDTA (sodium salt, dehydrate)	18.61	g
Distilled water	100	ml

Effective concentration to inhibit mettalo-proteases is 1 mM. Stable for months at 4°C.

2. Iodoacetic acid (IAA), 50 mM

Iodoacetic acid (sodium salt)	1.04	g
Distilled water	100	ml

Effective concentration to inhibit serine-proteases is 10-50 μM. Stable for months at -20°C. Decomposes slowly should be prepared freshly.

3. Phenylmethanesulphonyl fluoride (PMSF), 50 mM

Phenylmethanesulfonyl fluoride	0.87	g
Methanol	100	ml

Effective concentration to inhibit cysteine-proteases is 0.1-1 mM. Stable at least 9 months at 4°C.

-<u>For fungus killing</u>;

Merthiolate, 0.1% stock

Merthiolate (Thimerosol;Sigma)	1.0	g
Sodium tetraborate	0.74	g
Distilled water	100	ml

Store at 4°C in screwcapped dark brown bottle.

-For scraping yeast colonies;

0.85% Normal saline solution (NSS) +0.05% tween80

Sodium chloride	0.85	g
Distilled water	100	ml
Tween80	0.05	ml

Dissolve, disperse 3 ml in screwcapped-tube. Autoclave at 121°C 15 lbs for 15 min.

-For SDS-PAGE ;

1. **Stock acrylamide solution, 30% T 2.6% C**

Acrylamide	29.2	g
N',N'-bis-methylene-acrylamide	0.8	g
Distilled water up to	100	ml

Store in screwcapped dark brown bottle. Stable at least 1 month at 4°C.

2. **Ammonium persulfate (APS),** 10% stock

Ammonium persulfate	0.05	g
Distilled water	100	ml

Prepare fresh daily.

3. **Bromphenol blue tracking dye,** 0.1% stock

Bromphenol blue	0.1	g
Ethanol	100	ml

4. **Electrode reservoir buffer solution (5X Running buffer),** pH 8.3

Tris base	15	g
Glycine	72	g
SDS	5	g
Distilled water up to	1,000	ml

Do not adjust pH with acid or base. Store at 4°C.

5. Sample buffer (2X reducing buffer)

SDS	1.0	g
Glycerol	2.0	ml
0.1% Bromphenol blue	2.0	ml
1M Tris-HCl, pH 6.8	1.25	ml
2-β-mercaptoethanol	1.0	ml
Distilled water up to	10	ml

Store at 4°C and should be prepared fresh weekly.

6. Sodium dodecyl sulphate (SDS), 10% stock solution

SDS	1.0	g
Distilled water	10	ml

Store at room temperature and should be prepared fresh weekly.

7. 1.5 M Tris-HCl, pH 8.8 (separating gel buffer)

Tris base	18.15	g
Distilled water	60	ml

Dissolve and adjust to pH 8.8 with 5 N HCl, making up to 100 ml with distilled water. Store at 4°C.

8. 0.5 M Tris-HCl, pH 6.8 (stacking gel buffer)

Tris base	6.05	g
Distilled water	60	ml

Dissolve and adjust to pH 6.8 with 5 N HCl, making up to 100 ml with distilled water. Store at 4°C.

9. Separating gel monomer solution

	7.5% gel	10% gel
Distilled water	4.9 ml	4.0 ml
1.5 M Tris-HCl	2.5 ml	2.5 ml
Stock acrylamide (30%T 2.67%C)	2.5 ml	3.33 ml

10% SDS	0.1 ml	0.1 ml

Mix well and allow to stand about 5 min.

10% APS	50 µl	50 µl
TEMED	5 µl	5 µl
Total volume	10 ml	10 ml

Mix gently and use immediately.

10. Stacking gel monomer solution (4% T, 2.6% C)

Distilled water	6.1	ml
0.5 M Tris-HCl, pH 6.8	2.5	ml
Stock acrylamide	1.3	ml
10% SDS	0.1	ml

Mix well and allow to stand about 5 min.

10% APS	100	µl
TEMED	10	µl
Total volume	10	ml

Mix gently and use immediately.

11. Transfer buffer, pH8.3 (For nitrocellulose membrane)

Tris base	3.03	g
Glycine	14.4	g
Methanol	200	ml
Distilled deionized water up to	1,000	ml

Do not adjust pH with acid or base. Store at 4°C.

-For two-dimensional gel electrophoresis;

First dimension solution

1. First dimension acrylamide stock solution (30% T, 5.4% C)

Acrylamide	28.38	g
N',N'-bis-methylene acrylamide	1.62	g
Distilled water up to	100	ml

Dissolve completely and filter through Whatman no.1. Store at 4°C in screwcapped dark brown bottle. Stable at least 1 month.

2. First dimension sample buffer

9.5 M urea	5.7	g
2.0% Triton X-100	2.0	ml of 10% stock
5% β-mercaptoethanol	0.5	ml
1.6% Bio-Lyte 5/7 ampholyte	0.4	ml
0.4% Bio-Lyte 3/10 ampholyte	0.1	ml
Deionized distilled water up to	10	ml

Warm this solution in a shaking water bath of 30°C to dissolve urea. Aliquot into 0.5 ml of volume in Eppendorf tubes and store at -70°C.

3. First dimension sample overlay buffer

9 M urea	5.41	g
0.8% Bio-Lyte 5/7 ampholyte	0.2	ml
0.2% Bio-Lyte 3/10 ampholyte	0.05	ml
Bromphenol blue	0.25	ml of 0.1% stock
Deionized distilled water up to	10	ml

Warm this solution in a shaking water bath to dissolve urea at 30°C. Aliquot into 0.5 ml of volumes in Eppendorf tube and store at -70°C.

4. 10% Triton X-100, stock solution

Triton X-100	10	ml
Distilled water	100	ml

Deionized this solution with 5 g of AG 501-X8 ion exchange resin, and then filtered through Whatman no.1 filter paper. Store at room temperature.

5. Upper chamber buffer (100 mM NaCl)

NaOH	1	g
Distilled water	250	ml

Degas throughly for 30 min. Store at room temperature.

6. Lower chamber buffer (10 mM H_3PO_4)

Concentrate H_3PO_4	1.36	ml
Distilled water up to	2,000	ml

Degas throughly for 30 min. Store at room temperature.

7. First dimension gel monomer solution (IEF gel)

9.2 M urea	5.5	g
4% acrylamide	1.33	ml of stock acrylamide (30%T 5.4%C)
2.0% Triton X-100	2.0	ml of 10% stock
1.6% Bio-Lyte 5/7 ampholyte	0.4	ml Bio-Lyte 5/7
0.4% Bio-Lyte 3/10 ampholyte	0.1	ml Bio-Lyte 3/10
Distilled water	1.97	ml

Dissolved this solution by warming in a shaking water bath at 30°C. Degas the solution thoroughly for 15 min, and then add 10 µl of 10% APS (final concentration 0.01%) which was prepared freshly and 10 µl of TEMED that final concentration is 0.1%. Mix gently and use immediately.

Second dimension solution

1. SDS sample equilibrium buffer

0.0625 M Tris-HCl, pH 6.8	12.5	ml of 0.5M Tris-HCl, pH 6.8
2.3% SDS	23	ml of 10% SDS
5.0% β-mercaptoethanol	5	ml
10% Glycerol (w/v)	8	ml
Bromphenol blue	1.25	ml of 0.1% stock

Distilled water up to	100	ml

Store at 4°C and should be prepared fresh weekly.

2. Second dimension gel monomer solution

-12.5% separating gel solution

Distilled water	4.86	ml
1.5 M Tris-HCl, pH 8.8	3.75	ml
Stock acrylamide (30%T 2.67%C)	6.24	ml
10% SDS	0.15	ml

Mix well and allow to stand for 5 min, and then add

10% APS	100	µl
TEMED	10	µl
Total volume	15	ml

Mix gently and use immediately. This volume use for 1 mm of gel thickness per 2 gels.

-4% stacking gel solution

Distilled water	3.05	ml
0.5 M Tris-HCl, pH 6.8	1.25	ml
Stock acrylamide solution	0.65	ml
10% SDS	0.05	ml

Mix well and allow to stand for 5 min, and then add

10% APS	50	µl
TEMED	5	µl
Total volume	5	ml

Mix gently and use immediately

-<u>For glycoprotein detection</u>;

1. **TBS** (50mM Tris, 27 mM sodium chloride, pH 7.2)

Tris base	6.05	g
NaCl	1.6	g
Deionized distilled water	900	ml

Dissolve the reagents in ddH_2O. Adjust pH to 7.2 with 1 N HCl and fill ddH_2O up to 1,000 ml. Store at 4°C (for long term storage add 0.01 % NaN_3)

2. 200 mM Sodium Acetate Buffer, pH 5.5

A. Sodium acetate trihydrate	12.0	g
Deionized distilled water	440	ml
B. Glacial acetic acid	0.69	ml
Deionized distilled water	60	ml

Solution A. and B. were combined together. Check pH is 5.5 and then store at 4°C.

3. Color Development Buffer

Tris base	1.21	g
$MgCl_2.6H_2O$	1.01	g
NaCl	0.58	g
Deionized distilled water	90	ml

These reagents were dissolved in the water. Adjust pH to 9.5 with 0.1 N HCl and fill ddH_2O up to 100 ml. Store at 4°C.

4. PBS (9 mM sodium phosphate, 27 mM sodium chloride, pH 7.2)

Na_2HPO_4	575	mg
NaH_2PO_4	100	mg
NaCl	800	mg

Disslove reagents in 400 ml ddH_2O and adjust pH to 7.2 with 1N HCl or 1N NaOH, and then fill ddH_2O up to 500 ml. Store at 4°C.

5. 100 mM Sodium Acetate/5mM EDTA

EDTA (tetra sodium salt)	1.14	g
200 mM sodium acetate, pH 5.5	300	ml

Dissolve EDTA in sodium acetate solution and fill ddH_2O up to 600 ml. Store at 4°C.

6. **Dimethylformamide**, ready to use

-For lectin blotting;

1. **TBS** (150 mM NaCl, 10 mM Tris-HCl, pH 7.5)

NaCl	8.7	g
Tris-HCl	1.6	g
Distilled water	900	ml

Dissolve completely reagents in D.W. and adjust pH to 7.5 with 1N HCl or 1N NaOH. After that, fill D.W. up to 1,000 ml. Store at 4°C.

2. **TTBS**

TBS (pH 7.5) solution	100	ml
Tween-20	0.05	ml

Add Tween-20 in TBS and mix well. It should be prepared freshly before use.

3. **Blocking solution**

TTBS	100	ml
BSA	5	g

BSA was completely dissolved in TTBS by using magnetic stirrer. Store at 4°C.

4. **TBS** (0.2 M NaCl, 20 mM Tris-HCl, pH 7.5); for working substrate

NaCl	12	g
Tris-HCl	3.2	g
Distilled water	900	ml

Dissolve reagents in D.W. and adjust pH to 7.5 with 1N HCl or 1N NaOH, and then fill D.W. up to 1,000 ml. Store at 4°C.

5. Stock chromogen solution

4-chloro-1-naphthol	30	mg
Ice cold methanol	10	ml

Mixed the solution with vortex mixture and aliquot to 1 ml in microtubes. Store at –20 °C.

6. Working chromogenic substrate

TBS (for working substrate)	10	ml
Stock chromogen solution	2	ml

Mix well and add 6 µl of 30% H_2O_2 before using. It should be prepared freshly.

Pre-treatment of dialysis tubing

1. Select dialysis tubing and cut into lengths required.
2. Submerge in a solution of 2% sodium bicarbonate and 0.05% EDTA. Ensure sufficient volume is used to apply cover the dialysis tubing. Boil for 10 min. Ensure the dialysis tubing remains submerge by placing a conical flask partially filled with water on the top of tubing.
3. Discard the solution and boil for 10 min in distilled water. Repeat once more.
4. Cool and place into suitable solution to prevent microbial growth (e.g. 20% V/V ethanol or 0.1% sodium azide). Store at 4°C for up to 3 months.

IX. CURRICULUM VITAE

NAME Ms. Walla Poolsri

DATE OF BIRTH February 10, 1960

PLACE OF BIRTH Phitsanuloke, Thailand

Institution Attended:

1977	Certificate in Mathayom VI, Chalermkwant Stree School, Phitsanuloke, Thailand.
1978	Certificate in Clinical Laboratory Assistant, Faculty of Associated Medical Science, Mahidol University, Bangkok, Thailand.
1982	Certificate in Medical Laboratory Technician, The school of Medical Laboratory Technician, Department of Medical Science, Ministry of Public Health in Bangkok, Thailand.
1989	Bachelor of Education (B.Ed., Science-biology), Sri Nakharinwirot University Phitsanuloke, Phitsanuloke, Thailand.

Practical Experience:

1979-1984	Medical Laboratory Technician, Budachinaraj Hospital, Phitsanuloke, Thailand.
1985-1992	Medical Laboratory Technician, Regional Medical Science Center, Phitsanuloke.
1993-1994	Scientist, Somdedprayuparaj Rhomkao Hospital, Petchaboon, Thailand.
1995-	Instructor, Sirinthorn Public Health College Phitsanuloke, Phitsanuloke, Thailand.