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APPENDIX

1. Analysis of Antigen extracts by SDS-PAGE

7.5,10,15% separating gels

⇓ polymerize, overnight, RT

4% stacking gels

⇓ polymerize, 1 hr, RT

loading of samples and standards

⇓

electrophoresis in buffer pH 8.3

⇓ 40 mA/gel, 4 hr

staining by Coomassie brilliant blue or silver stain (for LPS)

⇓

photograph or gel drying

⇓

determination of molecular weight

2. Determination of Antibody titers by ELISA

coat plates with homologous KSCN antigen extract 1.0 µg/well

⇓ 4°c, overnight, washed with PBS-T

block non-specific binding sites with 3% BSA

⇓ 37°c, 1 hr, wash with PBS-T

add immune sera dilution $1:10^1$-$1:10^6$, 100 µl/well

⇓ 37°c, 1 hr, wash with PBS-T

add anti-rabbit Ig conjugate to HRP dilution 1:3,000, 100 µl/well

⇓ 37°c, 1 hr, wash with PBS-T

add substrate (OPD + H_2O_2), 100 µl/well

⇓ RT, 15-20 min, dark

stop reaction with 4 N sulfuric acid, 50 µl/well

⇓

read OD at 492 nm

3. Analysis of Antigen Extracts with Rabbit Immune Sera by Western Blot

KSCN antigen extract, capsule,OMP and LPS

⇓

separating by SDS-PAGE

⇓ 40 mA/gel, 4 hr

transfer to nitrocellulose membrane

⇓ 25 V, 2.5 hr

block non-specific binding with 3% BSA in TBS

⇓ 1 hr, RT

incubate with rabbit immune sera

⇓ 4°c, overnight

incubate with anti-rabbit Ig conjugated to HRP dilution 1:1,000

⇓ 2 hr, RT

incubate with substrate (4-chloro-1-napthol + H_2O_2)

⇓ 1 hr, RT

stop reaction by washing in distilled water

⇓

storage by photographing or wrapping in aluminum foil

4. Preparation of Media

4.1) Brain heart infusion broth (Difco, U.S.A)

Calf brain infusion	200.0	gm
Beef heart infusion	250.0	gm
Bacto proteose peptone	10.0	gm
Bacto dextrose	2.0	gm
Sodium chloride	5.0	gm
Disodium phosphate	2.5	gm
Distilled water to	1,000.0	ml

Steriled by autoclaving at 121°c, 15 lbs, 15 min

4.2) Tryptose blood agar (Difco, U.S.A)

Bacto tryptose	10.0	gm
Bacto beef extract	3.0	gm
Sodium chloride	5.0	gm
Bacto agar	15.0	gm
Distilled water to	1,000.0	ml

Sterile by autoclaving at 121°c, 15 lbs, 15 min. Cool the sterile media to 45-50°c. Aseptically add 5% sterile sheep blood. Mix well and dispense as desired.

5. Preparation of Reagents for Antigen Extracts

5.1) 0.5 M Potassium thiocyanate, 0.08 M Sodium chloride pH 6.3

Potassium thiocyanate	24.3	gm
Sodium chloride	2.4	gm
Distilled water to	500.0	ml

adjust pH to 6.3 before bring to final volume

5.2) 0.04 M Sodium chloride

Sodium chloride	1.3	gm
Distilled water to	500.0	ml

5.3) 0.15 M Sodium chloride

Sodium chloride	8.8	gm
Distilled water to	1,000.0	ml

5.4) 0.01 M Tris-hydrochloride, 0.32 M Sodium chloride pH 8.0

Tris-hydrochloride	1.6	gm
Sodium chloride	18.7	gm
Distilled water to	1,000.0	ml

adjust pH to 8.0 before bring to volume

5.5) 10 mM HEPE buffer pH 7.4

HEPE	1.2	gm
Distilled water to	500.0	ml

adjust pH to 7.4 before bring to volume

5.6) 2% Sakosyl in HEPE buffer pH 7.4

Sakosyl	2.0	gm
HEPE buffer pH 7.4	100.0	ml

5.7) TAE buffer (40 mM Tris-acetate pH 8.5, 2 mM EDTA)

Tris-hydrochloride	0.6	gm
EDTA	0.1	gm
Distilled water to	100.0	ml

5.8) Alkaline solution

SDS	3.0	gm
Trizmabase	0.6	gm
2 N Sodium hydroxide	6.4	ml
Distilled water to	100.0	ml

5.9) 3 M Sodium acetate pH 5.2

Sodium acetate	40.8	gm
Distilled water to	100.0	ml

5.10) 50 mM Tris-Hydrochloride pH 8.0, 100 mM Sodium acetate

Tris-Hydrochloride	0.8	gm
Sodium acetate	1.4	gm
Distilled water to	100.0	ml

6. Preparation of Reagents for SDS-PAGE

6.1) 30% Acrylamide-0.8% Bis-acrylamide

Acrylamide	30.0	gm
Bis-acrylamide	0.8	gm
Distilled water to	100.0	ml

filter and store up to 3 months at 4°c in the dark

6.2) 5x Running buffer pH 8.3 (0.025 M tris, 0.102 M glycine, 0.1% SDS)

Trizmabase	15.0	gm
Glycine	72.0	gm
SDS	5.0	gm
Distilled water to	1,000.0	ml

adjust pH to 8.3 before bring to volume

6.3) Stacking gel buffer pH 6.8 (0.5 M Tris-hydrochloride)

Trizmabase	6.0	gm
Distilled water to	100.0	ml

adjust pH to 6.8 with 6 N hydrochloric acid before bring to volume

6.4) Separating gel buffer pH 8.8 (1.5 M Tris-hydrochloride)

Trizmabase	18.2	gm
Distilled water to	100.0	ml

adjust pH to 8.8 with 6 N hydrochloric acid before bring to volume

6.5) 10% Sodium dodecyl sulfate (SDS)

SDS	10.0	gm
Distilled water to	100.0	ml

store at room temperature

6.6) 10% Ammonium persulfate

Ammonium persulfate	0.1	gm
Distilled water to	1.0	ml

use fresh, do not store

6.7) Sample buffer (0.06 M Tris-hydrochloride pH 6.8, 10% Glycerol, 2% SDS, 5% Mercaptoethanol, 0.025% Bromophenol blue)

0.5 M Tris-hydrochloride pH 6.8	1.2	ml
Glycerol	1.0	ml
10% SDS	2.0	ml
2-Mercaptoethanol	0.5	ml
0.5% Bromophenol blue	0.5	ml
Distilled water	2.0	ml

6.8) Staining solution (0.1% Coomassie brilliant blue, 40% Methanol, 10% Acetic acid)

Coomassie brilliant blue R-250	1.0	gm
Methanol	400.0	ml
Acetic acid	100.0	ml
Distilled water	500.0	ml

filter before use

6.9) De-staining solution (40% Methanol, 10% Acetic acid)

Methanol	400.0	ml
Acetic acid	100.0	ml
Distilled water to	500.0	ml

7. Preparation of Reagents for ELISA

7.1) 0.05 M Carbonate- Bicarbonate buffer pH 9.6 (coating buffer)

Sodium carbonate	0.8	gm
Sodium hydrogen carbonate	1.5	gm
Distilled water to	500.0	ml

adjust pH to 9.6 before bring to volume

7.2) Citrate-Phosphate buffer pH 5.0 (substrate buffer)

Citric acid	9.3	gm
Sodium hydrogen phosphate	18.3	gm
Thimerosol	0.1	gm
Distilled water to	1,000.0	ml

adjust pH to 5.0 before bring to volume

7.3) Phosphate buffer saline pH 7.4 with Tween 20 (Washing buffer)

Sodium chloride	8.0	gm
Potassium dihydrogen phosphate	0.2	gm
Sodium hydrogen phosphate	2.9	gm
Potassium chloride	0.2	gm
Thimerosol	0.1	gm
Tween 20	0.5	ml

Distilled water to 1,000.0 ml, adjust pH 7.4 before bring to volume

7.4) 1% Bovine serum albumin in PBS-T (diluent)

Bovine serum albumin	1.0	gm
PBS-T	100.0	ml

7.5) 3% Bovine serum albumin in PBS-T (blocking solution)

Bovine serum albumin	3.0	gm
PBS-T	100.0	ml

7.6) 4 N Sulfuric acid

98% Sulfuric acid	54.4	ml
Distilled water to	500.0	ml

8. Preparation of Reagents for Western Blot

8.1) Blotting buffer pH 9.2 (48 mM Tris, 39 mM Glycine, 20% Methanol, 0.0375% SDS)

Trizmabase	5.8	gm
Glycine	2.9	gm
SDS	0.04	gm
Methanol	200.0	ml
Distilled water to	1,000.0	ml

do not adjust pH

8.2) Tris buffer saline pH 7.4 (50 mM Tris-hydrochloride, 0.85% Sodium chloride)

Tris-hydrochloride	7.9	gm
Sodium chloride	8.5	gm
Thimerosol	0.1	gm
Distilled water to	1,000.0	ml

adjust pH to 7.4 before bring to volume

8.3) Tris buffer saline pH 9.5 (0.1 M Trizmabase, 0.85% Sodium chloride)

Trizmabase	12.1	gm
Sodium chloride	8.5	gm
Distilled water to	1,000.0	ml

adjust pH to 9.5 before bring to volume

8.4) 4-Chloro-1-napthol peroxidase substrate

4-Chloro-1-napthol	0.03	gm
Methanol	10.0	ml

8.5) 0.1% Amido black

Amido black	0.1	gm
Methanol	40.0	ml
Acetic acid	10.0	ml
Distilled water to	100.0	ml

BIOGRAPHY

Miss Malai Luempol was born on August 31, 1966 in Mahasarakham, Thailand. She graduated with a Bachelor of Science in Zoology from the Faculty of Science at Chulalongkorn University in 1989.

Miss Malai is currently working as a scientist in the Department of Microbiology, Faculty of Pharmaceutical Science, Chulalongkorn University.