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APPENDICES

1. Media for bacterial culture

1.1 Ashdown's agar

Tryptic Soy Broth	10 g
Agar	15 g
Crystal violet 0.1%	5 mL
(incubate at least 1 week before use)	
Neutral red 1%	5 mL
Distilled water	910 mL

Autoclave at 120 °C for 15 min. Add glycerol 40 mL and gentamicin to a final concentration of 5mg/L, and dispense into Petri dishes.

1.2 Tryptic Soy Broth

Tryptic Soy Broth	1.5 g
Distilled water	50 mL

Autoclave at 120 °C for 15 min.

2. Reagents for ELISA

2.1 Coating buffer for ELISA

Na ₂ CO ₃	1.59 g.
NaHCO ₃	2.93 g.

Add distilled water to final volume of 1,000 ml and adjust pH to 9.6

2.2 Washing buffer (10X) for ELISA

NaCl	90 g.
Tween20	5 ml
DW to	1,000 ml

Dilute 1:10 with DW before use (washing buffer (10X)).

2.3 Incubation buffer (1X) for ELISA

NaCl	8.9 g.
Na ₂ HPO ₄	1.28 g.
NaH ₂ PO ₄ ·2H ₂ O	0.15 g.
Tween20	0.5 ml
DW to	1,000 ml

Use for diluting Ab and conjugate.



2.4 0.15 M Phosphate buffer saline (PBS) pH 7.2

Na ₂ HPO ₄	8.09	g
KH ₂ PO ₄	2.45	g
NaCl	4.4	g
NaN ₃	1	g

Add distilled water to final volume of 1,000 ml and adjust pH to 7.2 with 1 N

HCl or 1 N NaOH

2.5 Substrate Reagent Pack (R&D system) no. DY 9999

Color reagent A (peroxidase solution)	5	ml
Color reagent B (chromogen solution)	5	ml

2.6 Stop solution (2 M H₂SO₄)

H ₂ SO ₄	100	ml
DW to	900	ml

2.7 Block buffer (5% skim milk)

Skim milk	5	g
Incubation buffer (1X) to	100	ml

3. Reagents for PCR master mixed

3.1 Master mixed 1 reaction

Includes: Distilled water	26.5	μl
10X PCR buffer	5	μl
50 mM MgCl ₂ (2mM)	2	μl
dNTP mix (1mM)	2.5	μl
Primer webG_for primer (1μM)	2.5	μl
Primer webG_rev primer (1μM)	2.5	μl
Taq DNA polymerase (5U)	4	μl

4. Reagents for agarose gel electrophoresis**4.1 1.5% agarose gel**

Agarose gel	5 g
1X TBE	100 ml

4.2 10X TBE buffer (pH 8.0)

Tris-base	45 g
Boric acid	27.5 g
0.5 M Na EDTA (pH 8.0)	20 ml
Distilled water to	500 ml

Store at room temperature.

4.3 Loading dye

Bromphenol blue	0.125 g
Glycerol	15 ml
10X TBE buffer	30 ml
Distilled water to	50 ml

4.4 Ethidium bromide (10 mg/ml)

Ethidium bromide	1 g
Distilled water	100 ml

RESEARCH PRESENTATIONS

1. Nuttiya Srisurat, Rasana Wongratanacheewin, Unchalee Tattawasart, Suwuna Nontapa and Surasakdi Wongratanacheewin, (2009, March 20-23). **Kinetics of *Burkholderia pseudomallei* and antibody responses in blood of acute and chronic infected BALB/c mice.** Ministry of public health conference. IMPACT Muang Thong Thani. Bangkok, Thailand. (Oral presentation)

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