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APPENDICES

APPENDIX A

CHEMICAL PREPARATION

A.1 Blocking buffer for indirect ELISA (PBS-Tween, pH 7.4 containing 1.5% BSA)

NaCl	8	grams
KH ₂ PO ₄	0.24	grams
Na ₂ HPO ₄ .12H ₂ O	2.9	grams
KCl	0.2	grams
Tween 20	0.5	mL
Bovine serum albumin (BSA)	15	grams

Mix all reagents and dissolve in 800 mL distilled water.

Adjust pH to 7.4, make the volume to 1 liter.

A.2 Coating buffer for indirect ELISA (Carbonate/Bicarbonate pH 9.6)

Na ₂ CO ₃	1.59	grams
NaHCO ₃	2.93	grams

Both reagents were mixed dissolved in 1 liter distilled water.

A.3 Washing buffer for indirect ELISA (PBS-Tween, pH 7.4)

NaCl	8	grams
KH ₂ PO ₄	0.24	grams
Na ₂ HPO ₄ .12H ₂ O	2.9	grams
KCl	0.2	grams
Tween 20	0.5	mL

Mix all reagents and dissolve in 800 mL distilled water.

Adjust pH to 7.4, make the volume to 1 liter.

A.4 Modified Sheather solution

Sucrose	500	grams
Phenol	5	mL
Tap water	350	mL

A.5 Phosphate buffer saline (PBS buffer, pH 7.4)

NaCl	8	grams
KH ₂ PO ₄	0.24	grams
Na ₂ HPO ₄	1.44	grams
KCl	0.2	grams

Mix all reagents and dissolve in 800 mL distilled water.

Adjust pH to 7.4, make the volume to 1 liter.

A.6 100 mM sodium phosphate buffer, pH 7.6

NaHPO ₄ .H ₂ O	13.8	grams
Na ₂ HPO ₄	14.2	grams

Both reagents were mixed and dissolved in 800 mL distilled water.

Adjust pH to 7.6, make the volume to 1 liter.

APPENDIX B

EXPERIMENTAL RESULTS

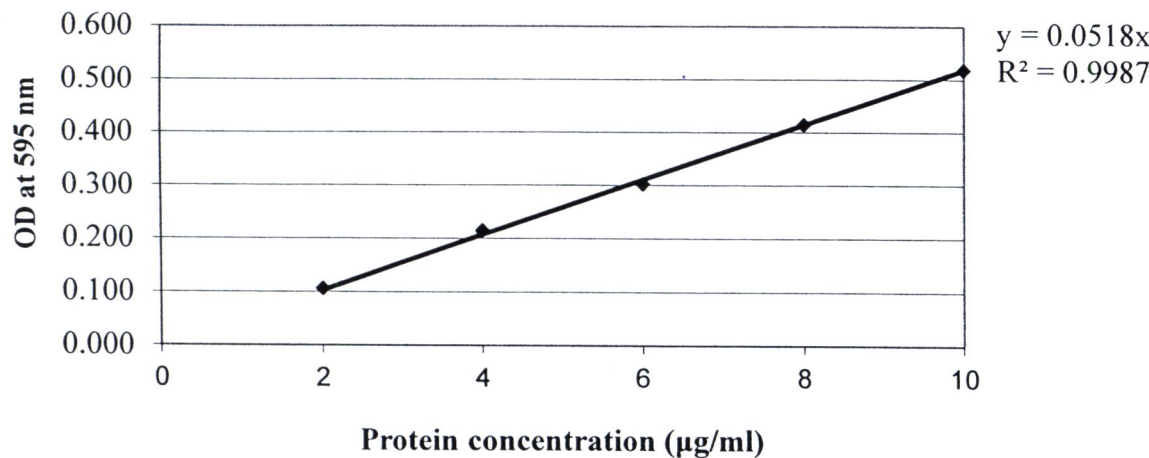


Figure B.1 Standard curve for Bradford protein assay

Table B.1 Titer determination of extracted IgY in pooled fresh egg from week 13 after first immunization

Samples	Dilutions	Protein Conc. (ug/mL)	O.D at 450 nm			Av. OD
Blank	-	-	0.063	0.065	0.064	0.064
No Ag.	1:160	35	0.410	0.464	0.421	0.432
Negative Control (Extracted IgY against adipocyte membrane)	1:160	35	0.647	0.626	0.641	0.638
	1:320	17.5	0.505	0.497	0.511	0.504
	1:640	8.75	0.361	0.364	0.348	0.358
	1:1280	4.38	0.238	0.241	0.249	0.243
	1:2560	2.12	0.161	0.175	0.186	0.174
No Ag.	1:160	35	1.218	1.169	1.173	1.187
Extracted anti-chicken coccidia IgY	1:160	35	2.097	2.097	2.041	2.078
	1:320	17.5	1.549	1.561	1.541	1.550
	1:640	8.75	1.012	0.991	1.001	1.001
	1:1280	4.38	0.557	0.544	0.578	0.560
	1:2560	2.12	0.314	0.307	0.326	0.316
No IgY	-	-	0.074	0.077	0.070	0.074

Table B.2 Titer determination of anti-chicken coccidia IgY in pooled fresh yolk from week 14 -21 after first immunization

Samples	Dilutions	OD at 450 nm			Av. OD
Blank I	-	0.053	0.053	0.052	0.053
Negative Control (IgY against adipocyte membrane protein)	No Ag (1 :160)	0.831	0.815	0.826	0.824
	1 : 160	1.395	1.408	1.371	1.391
	1 : 320	0.940	0.922	0.911	0.924
	1 : 640	0.673	0.619	0.629	0.640
	1 :1280	0.393	0.404	0.401	0.399
	1 : 2560	0.229	0.225	0.222	0.225
Week 6	No Ag (1 :160)	1.302	1.298	1.286	1.295
	1 : 160	2.206	2.228	2.219	2.218
	1 : 320	1.626	1.660	1.643	1.643
	1 : 640	1.062	1.049	1.053	1.055
	1 :1280	0.634	0.647	0.628	0.636
	1 : 2560	0.388	0.396	0.380	0.388
Week 14	No Ag (1 :160)	1.142	1.137	1.090	1.123
	1 : 160	2.239	2.272	2.296	2.269
	1 : 320	1.664	1.665	1.660	1.663
	1 : 640	1.014	1.056	1.044	1.038
	1 :1280	0.640	0.612	0.601	0.618
	1 : 2560	0.381	0.370	0.386	0.379
Blank II	-	0.050	0.050	0.050	0.050
Week 15	No Ag (1 :160)	1.596	1.581	1.556	1.578
	1 : 160	2.201	2.176	2.206	2.194
	1 : 320	1.560	1.568	1.569	1.566
	1 : 640	1.128	1.155	1.148	1.144
	1 :1280	0.783	0.781	0.747	0.770
	1 : 2560	0.401	0.403	0.405	0.403
Week 16	No Ag (1 :160)	1.490	1.496	1.528	1.505
	1 : 160	2.199	2.235	2.187	2.207
	1 : 320	1.546	1.519	1.519	1.528
	1 : 640	1.009	0.967	0.966	0.981
	1 :1280	0.584	0.571	0.588	0.581
	1 : 2560	0.341	0.358	0.341	0.347
Week 17	No Ag (1 :160)	0.858	0.854	0.856	0.856
	1 : 160	1.981	1.993	1.988	1.987
	1 : 320	1.366	1.359	1.376	1.367
	1 : 640	0.979	0.976	0.986	0.980
	1 :1280	0.599	0.586	0.584	0.590
	1 : 2560	0.331	0.349	0.375	0.352

Table B.2 Titer determination of anti-chicken coccidia IgY in pooled fresh yolk from week 14 - 21 after first immunization (Cont.)

Samples	Dilutions	OD at 450 nm			Av. OD
Blank III	-	0.055	0.053	0.054	0.054
Week 18	No Ag (1 :160)	0.912	0.936	0.928	0.925
	1 : 160	1.983	1.982	1.972	1.979
	1 : 320	1.414	1.422	1.393	1.410
	1 : 640	0.968	0.950	0.921	0.946
	1 :1280	0.554	0.572	0.585	0.570
	1 : 2560	0.320	0.330	0.342	0.331
Week 19	No Ag (1 :160)	1.159	1.192	1.242	1.198
	1 : 160	1.868	1.876	1.888	1.877
	1 : 320	1.357	1.346	1.320	1.341
	1 : 640	0.839	0.854	0.853	0.849
	1 :1280	0.513	0.532	0.539	0.528
	1 : 2560	0.296	0.312	0.327	0.312
Week 20	No Ag (1 :160)	1.074	1.063	1.053	1.063
	1 : 160	1.816	1.831	1.805	1.817
	1 : 320	1.256	1.234	1.231	1.240
	1 : 640	0.781	0.747	0.771	0.766
	1 :1280	0.408	0.413	0.396	0.406
	1 : 2560	0.206	0.204	0.211	0.207
Week 21	No Ag (1 :160)	0.704	0.723	0.737	0.721
	1 : 160	1.592	1.623	1.598	1.604
	1 : 320	1.021	1.008	1.014	1.014
	1 : 640	0.620	0.616	0.597	0.611
	1 :1280	0.328	0.330	0.323	0.327
	1 : 2560	0.185	0.187	0.191	0.188

Table B.3 Titer determination of anti-chicken coccidia IgY in pooled dried yolk from week 14 -21 after first immunization

Sample	Dilutions	OD at 450 nm			Av. OD
Blank I	-	0.055	0.053	0.052	0.053
Negative Control IgY against adipocyte membrane protein)	No Ag (1 :160)	1.024	1.012	1.040	1.025
	1 : 160	1.507	1.513	1.535	1.518
	1 : 320	1.126	1.140	1.148	1.138
	1 : 640	0.716	0.722	0.739	0.726
	1 :1280	0.502	0.504	0.496	0.501
	1 : 2560	0.302	0.298	0.289	0.296
Week 12	No Ag (1 :160)	1.721	1.736	1.744	1.734
	1 : 160	2.414	2.417	2.439	2.423
	1 : 320	1.864	1.889	1.882	1.878
	1 : 640	1.464	1.458	1.488	1.470
	1 :1280	1.069	1.080	1.074	1.074
	1 : 2560	0.666	0.640	0.652	0.653
Week 13	No Ag (1 :160)	1.688	1.669	1.677	1.678
	1 : 160	2.348	2.379	2.363	2.363
	1 : 320	1.859	1.837	1.845	1.847
	1 : 640	1.435	1.430	1.437	1.434
	1 :1280	1.019	1.023	1.048	1.030
	1 : 2560	0.630	0.610	0.621	0.620
Week 14	No Ag (1 :160)	1.517	1.540	1.531	1.529
	1 : 160	2.271	2.244	2.262	2.259
	1 : 320	1.742	1.722	1.738	1.734
	1 : 640	1.287	1.257	1.266	1.270
	1 :1280	0.869	0.890	0.888	0.882
	1 : 2560	0.547	0.560	0.538	0.548
Blank II	-	0.051	0.052	0.054	0.052
Week 15	No Ag (1 :160)	1.791	1.779	1.798	1.789
	1 : 160	2.303	2.317	2.331	2.317
	1 : 320	1.882	1.878	1.857	1.872
	1 : 640	1.350	1.328	1.339	1.339
	1 :1280	0.974	0.969	0.955	0.966
	1 : 2560	0.549	0.557	0.574	0.560
Week 16	No Ag (1 :160)	1.568	1.548	1.572	1.563
	1 : 160	2.238	2.268	2.244	2.250
	1 : 320	1.769	1.744	1.762	1.758
	1 : 640	1.264	1.268	1.284	1.272
	1 :1280	0.928	0.933	0.941	0.934
	1 : 2560	0.539	0.524	0.532	0.532

Table B.3 Titer determination of anti-chicken coccidia IgY in pooled dried yolk from week 14 -21 after first immunization (Cont.)

Sample	Dilutions	OD at 450 nm			Av. OD
Week 17	No Ag (1 :160)	1.420	1.411	1.433	1.421
	1 : 160	2.146	2.151	2.175	2.157
	1 : 320	1.621	1.611	1.637	1.623
	1 : 640	1.239	1.247	1.251	1.246
	1 :1280	0.854	0.877	0.859	0.863
	1 : 2560	0.471	0.483	0.489	0.481
Week 18	No Ag (1 :160)	1.536	1.532	1.540	1.536
	1 : 160	2.238	2.220	2.240	2.233
	1 : 320	1.760	1.738	1.749	1.749
	1 : 640	1.332	1.322	1.346	1.333
	1 :1280	0.930	0.922	0.964	0.939
	1 : 2560	0.536	0.544	0.534	0.538
Blank III	-	0.052	0.054	0.054	0.053
Week 19	No Ag (1 :160)	1.355	1.339	1.351	1.348
	1 : 160	2.078	2.067	2.084	2.076
	1 : 320	1.679	1.686	1.659	1.675
	1 : 640	1.177	1.165	1.189	1.177
	1 :1280	0.789	0.763	0.749	0.767
	1 : 2560	0.449	0.468	0.435	0.451
Week 20	No Ag (1 :160)	1.132	1.144	1.150	1.142
	1 : 160	1.880	1.862	1.866	1.869
	1 : 320	1.324	1.340	1.332	1.332
	1 : 640	0.912	0.934	0.940	0.929
	1 :1280	0.518	0.528	0.536	0.527
	1 : 2560	0.292	0.288	0.296	0.292
Week 21	No Ag (1 :160)	1.199	1.184	1.207	1.197
	1 : 160	1.845	1.863	1.855	1.854
	1 : 320	1.311	1.332	1.307	1.317
	1 : 640	0.915	0.923	0.930	0.923
	1 :1280	0.509	0.521	0.513	0.514
	1 : 2560	0.287	0.295	0.281	0.288

CURRICULUM VITAE

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SCHOLARSHIP	The Thailand Research Fund (TRF) during 2008- 2009
PUBLICATION	Intanorat, T., Poomputsa, K., Akeprathumchai, S., Mekvichitsaeng, P., and Techkarnjanarak, S., 2010, “Production of Anti-chicken coccidia IgY”, The Eight Prince of Songkla Engineering Conference (PEC-8): Bioengineering for Medical , April 22 nd -23 rd 2010, Prince of Songkla University, Songkla, Thailand.

King Mongkut's University of Technology Thonburi
Agreement on Intellectual Property Rights Transfer for Postgraduate Students

Date 1 November 2011

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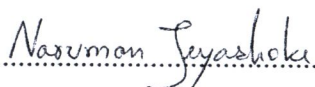
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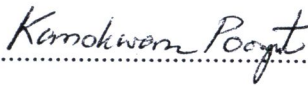
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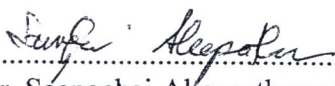
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Signature..........Witness
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Signature..........Witness
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