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APPENDICES

APPENDIX A

Reagent for laboratory experiment

1. Reagents for immunohistochemical staining

1.1 Phosphate buffer saline (PBS), pH 7.0-7.2

Stock solution A

0.2 M $NaH_2PO_4H_2O$	27.6	g
Distilled water	1,000	ml

Stock solution B

0.2 M $NaHPO_4$	28.4	g
Distilled water	1,000	ml

Working solution, 0.01 M PBS

Solution A	23	ml
Solution B	77	ml
Distilled water	1,900	ml
NaCl	18	ml

1.2 0.01 M PBS + 0.01% Tween 20

0.01 M PBS	1,000	ml
Tween 20	10	ml

1.3 1% Skim milk in PBS

Skim milk	1.0	g
0.01 M PBS	100	ml

1.4 Glycerol in PBS for mounting slide

Glycerol : PBS (1 : 4 volume/volume)

2. Reagents for nitrate/nitrite (NOx) determination

2.1 Griess reagent

0.1% N-(1-Naphthyl) Ethylenediamine Dihydrochloride (NEDD) solution

NEDD	0.2	g
Deionized water	200	ml

2% Sulfanilamide (SULF) solution

SULF	2.0	g
5% HCl	100	ml

Both solutions were stored at 4 °C in the dark bottle.

2.2 Saturated Vanadium trichloride (VCl_3) solution

VCl_3	0.8	g
1M HCl	100	ml

The solution was filtered using filter paper and stored at 4°C for less than two weeks

3. Reagents for malondialdehyde (MDA) determination

3.1 20 mM Thiobarbituric acid (TBA) in 15% Trichloroacetic acid

15% Trichloroacetic acid

Trichloroacetic acid	75	g
Deionized water	500	ml

20 mM TBA

TBA	1.44	g
15% Trichloroacetic acid	500	ml

3.2 n-Butanol in pyridine (15 : 1 volume/volume)

n-Butanol	900	ml
Pyridine	60	ml

3.3 7.2% Butylated hydroxytoluene (BHT)

BHT	7.2	g
95% Ethanol	100	ml

3.4 1,1,3,3-tetraethoxypropane (TEP) stock

TEP	50	µl
40% Ethanol	25	ml

3.5 8.1% Sodium dodecyl sulfate (SDS)

SDS	8.1	g
Deionized water	100	ml

4. Reagents for 8-hydroxy-2-deoxy Guanosine (8-oxodG) determination

4.1 EIA buffer

EIA buffer concentrate	10	ml
UltraPure water	90	ml

4.2 Wash buffer

Wash buffer concentrate	5.0	ml
UltraPure water	2000	ml
Tween 20	1.0	ml

4.3 Tracer

8- hydroxy-2-deoxy Guanosine AChE Tracer	100	dtn
EIA buffer	6.0	ml

4.4 Antibody

Goat Anti-Mouse IgG Coated Plate	1.0	plate

5. Reagents for ferric reducing antioxidant power (FRAP) measurement

5.1 300 mM Acetate buffer (pH 3.6)

Sodium acetate trihydrate	1.87	g
Glacial acetic acid	16	ml
Deionized water adjust to	1,000	ml

5.2 10 mM 2,4,6-Tripyridyl-s-triazine (TPTZ)

TPTZ	0.031	g
40 mM HCl	10	ml

5.3 20 mM Ferric chloride hexahydrate ($FeCl_3.6H_2O$)

$FeCl_3.6H_2O$	0.054	g
Deionized water	10	ml

6. Reagents for SDS polyacrylamide gel electrophoresis

6.1. 20% Sodium dodecyl sulphate (SDS)

SDS	20	g
Distilled water	100	ml
Stored at room temperature		

6.2 10x SDS electrophoresis buffer

(0.25 M Tris base, 2.5 M Glycine)

Tris base	30.29	g
Glycine	187.67	g

Distilled water adjust to 1000 ml

The pH of this solution was adjusted to 8.3 and stored at room temperature.

6.3 1x SDS electrophoresis buffer

(0.025M Tris base, 0.192M Glycine, 0.1% SDS)

-10x SDS electrophoresis buffer 100 ml

-20% SDS 5.0 ml

-Distilled water adjust to 1000 ml

6.4 Acrylamide solution

(30% acrylamide, 0.8% N, N'-methylenebisacrylamide)

Acrylamide 30 g

N, N'-methylenebisacrylamide 0.8 g

Distilled water 100 ml

Stored at 4 °C in the dark bottle

6.5 10% Ammonium persulfate solution

Ammonium persulfate 0.05 g

Distilled water 0.5 ml

6.6 4x Separating buffer

(1.5 M Tris-HCl, pH 8.8, 0.4% SDS)

Tris base 9.08 g

20% SDS 1.0 ml

Distilled water adjust to 100 ml

The pH of this solution was adjusted to 8.8 and stored at room temperature.

6.7 4x Stacking buffer

(0.5 M Tris-HCl, pH 6.8, 0.4% SDS)

Tris base 6.055 g

20% SDS 2.0 ml

Distilled water adjust to 100 ml

The pH of this solution was adjusted to 6.8 and stored at room temperature.

6.8 12% Separating gel

30% Acrylamide/Bis	3.2	ml
Separating buffer	2.0	ml
TEMED	10	µl
10% Ammonium persulphate	50	µl
Distilled water	2.8	ml

6.9 5% Stacking gel

30% Acrylamide/Bis	0.41	ml
Stacking buffer	0.8	ml
TEMED	8.0	µl
10% Ammonium persulphate	40	µl
Distilled water	1.95	ml

7. Reagent for western blotting

7.1 Liver homogenate extraction buffer (pH 7.4)

50 mM Tris-HCl pH 7.4	3.0	g
0.1% SDS	0.5	g
1% Triton X-100	5.0	ml
150 mM $NaCl_2$	4.383	g
Distilled water adjust to	500	ml

7.2 Nuclear extraction buffer

7.2.1 Cytoplasmic extraction buffer

10 mM HEPES pH 7.9	1.0	ml
1.5 mM KCl	75	µl
10 mM $MgCl_2$	2.0	ml
0.5 mM DTT	50	µl
0.5% NP-40	25	µl
0.5 mM phenylmethylsulfonyl fluoride (PMSF)	60	µl

7.2.2 Nuclear extraction buffer

20 mM HEPES pH 7.9	1.0	ml
25% glycerol	12.5	ml
1.5 mM $MgCl_2$	150	µl
420 mM NaCl	4.2	ml

0.5 mM DTT	5.0	µl
0.5% NP-40	20	µl
0.2 mM EDTA	20	µl
0.5 mM PMSF	12	µl

7.3 4x Sample buffer

(40% glycerol, 20% 2-mercaptoethanol, 8% SDS in Tris-HCl)

Glycerol	4.0	ml
2-mercaptoethanol	2.0	ml
SDS	0.4	g
0.5 M Tris-HCl, pH 6.8	5.0	ml
Bromophenol blue	0.0001	g

The pH of this solution was adjusted to 6.8 and stored at 4 °C in the dark bottle.

7.4 10x Transfer buffer

Tris-base	58.15	g
Glycine	29.3	g
SDS	3.75	g
Distilled water	1000	ml

The pH of this solution was adjusted to 8.4 and stored at RT

7.5 Working transfer buffer

10x Transfer buffer	100	ml
Methanol	200	ml
Distilled water	700	ml

8. Reagents for RNA isolation and RT-PCR analysis

8.1 Total RNA extraction

Trizol reagent	1,500	µl
Chloroform	300	µl
Isopropanol	750	µl
70% Ethanol	750	µl
0.05% DEPC	210	µl

8.2 10x TBE buffer

	Tris Base	107.8	g
	Boric acid	55	g
	EDTA	7.4	g
	Distilled water	1000	ml

8.3 2% Agarose gel

	Agarose	0.6	g
	1x TBE buffer	30	ml

8.4 Polymerase chain reaction

	10x buffer	2.0	µl
	50 mM $MgCl_2$	1.0	µl
	10 mM dNTP mix	2.0	µl
	Platinum Taq	0.1	µl
	Forward primer (5 µM)	2.0	µl
	Reverse primer (5 µM)	2.0	µl
	Distilled water	6.0	µl

APPENDIX B

Letter of Commendation for Presentation Awards

Certificate of Appreciation

presented to

Lakhanawan Charoensuk

for **an outstanding oral presentation**

In RGJ Seminar Series LXXV

"Biomedical Sciences: Research for Healthy Society"

Faculty of Medicine, Khon Kaen University

October 11th, 2010

Chairman of the Organizing Committee

RGJ Network Coordinator

HEDNet

บัณฑิตวิทยาลัย มหาวิทยาลัยขอนแก่น
และ
เครือข่ายอุดมศึกษาภาคตะวันออกเฉียงเหนือตอนบน

มอบเกียรติบัตรนี้ไว้เพื่อแสดงว่า

นางสาวลักขณาวัลย์ เจริญสุข

ได้นำเสนอผลงานวิจัยระดับบัณฑิตศึกษา แบบบรรยาย

ระดับปริญญาโท กลุ่มวิทยาศาสตร์สุขภาพ

ในการประชุมวิชาการเสนอผลงานวิจัยระดับบัณฑิตศึกษา ครั้งที่ 12

ณ มหาวิทยาลัยขอนแก่น

วันที่ 28 มกราคม พ.ศ.2554

(รองศาสตราจารย์ ดร.ลำปาง แม่นมาตย์)

คณบดีบัณฑิตวิทยาลัย มหาวิทยาลัยขอนแก่น

บัณฑิตวิทยาลัย มหาวิทยาลัยขอนแก่น

มอบเกียรติบัตรนี้ไว้เพื่อแสดงว่า

นางสาวลักขณาวัลย์ เจริญสุข

หลักสูตรวิทยาศาสตรมหาบัณฑิต สาขาวิชาปรสิตวิทยา

เป็นผู้สร้างชื่อเสียงให้กับมหาวิทยาลัยขอนแก่น ประจำปีการศึกษา 2553

โดยได้รับรางวัล outstanding oral presentation in RGJ Seminar Series LXXV

จาก การประชุมวิชาการทาง RGJ-Seminar Series LXXV (Biomedical Sciences : Research for Health Society) วันที่ 11 ตุลาคม 2553 ณ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

ให้ไว้ ณ วันที่ 28 มกราคม พ.ศ. 2554

(รองศาสตราจารย์ ดร. ลำปาง แม่นมาตย์)

คณบดีบัณฑิตวิทยาลัย

RESEARCH PUBLICATIONS

1. **Charoensuk, L.**, Pinlaor, P., Prakobwong, S., Hiraku, Y., Laothong, U., Ruangjirachuporn, W., Yongvanit, P. and **Pinlaor, S*.** Curcumin induces a nuclear factor-erythroid 2-related factor 2-driven response against oxidative and nitrative stress after praziquantel treatment in liver fluke-infected hamsters. *Int J Parasitol*, 2011, In-press.

2. **Lakhanawan Charoensuk**, Porntip Pinlaor, Suksanti Prakobwong, Yusuke Hiraku, Umawadee Laothong, Wipaporn Ruangjirachuporn, Puangrat Yongvanit, Somchai Pinlaor. Curcumin induces Nrf2-driven stress response against oxidative and nitrative stress after praziquantel-treated liver fluke-infection. (*Oral presentation*) The RGJ Seminar Series LXXV " Biomedical Sciences: Research for Healthy Society"; 11 October 2010 Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

3. **Lakhanawan Charoensuk**, Porntip Pinlaor, Wipaporn Ruangjirachuporn, Puangrat Yongvanit, Somchai Pinlaor. Protective effect of curcumin against adverse effect after praziquantel treatment in liver fluke-infected hamsters. (*Oral presentation*) The 12th Graduate Research Conference 2011; 28 January 2011, Khon Kaen University, Khon Kaen, Thailand.

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