



**APPENDICES**

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

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## APPENDIX A

List of the chemicals and materials used in this study. They were analytical grade unless otherwise stated.

Chemicals and materials	Source
Acrylamide	Bio Basic Inc., Toronto, Canada
Ammonium persulfate	Bio Basic Inc., Toronto, Canada
Autoradiographic film	Eastman Kodak Company, Rochester, NY, USA
BCA protein assay kit	Pierce, Rockford, IL, USA
Bis-acrylamide	Bio Basic Inc., Toronto, Canada
Bovine serum albumin	Sigma Chemical Co., St. Louis, MO, USA
Cling film	M.M.P. Packaging Group Inc., Bangkok, Thailand
COX-1 protein standard	Cayman Chemical, Ann Arbor, MI, USA (Purified from ram seminal vesicles, Catalog No. 60100)
COX-2 protein standard	Cayman Chemical, Ann Arbor, MI, USA (Purified from sheep placenta, Catalog No. 60120)
Developer	Eastman Kodak Company, Rochester, NY, USA
Ethanol	Merck, Darmstadt, Germany
ECL Western Blotting detection kit	Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK
Fixer	Eastman Kodak Company, Rochester, NY, USA
Glycerol	Bio Basic Inc., Toronto, Canada
Glycine	Research Organics Inc., St. Cleveland, OH, USA
Goat anti-mouse IgG conjugated HRP	Dako, Glostrup, Denmark

Hydrochloric acid	Merck, Darmstadt, Germany
2-mercaptoethanol	Bio Basic Inc., Toronto, Canada
Methanol	Fisher Scientific, Loughborough, Leicestershire, UK
Mouse anti-actin monoclonal antibody	Cayman Chemical, Ann Arbor, MI, USA (Synthetic actin C-terminal peptide used as antigen, Catalog No. A4700)
Mouse anti-COX-1 monoclonal antibody	Sigma Chemical Co., St. Louis, MO, USA (Purified ovine COX-1 used as antigen, Catalog No. 160110)
Mouse anti-COX-2 monoclonal antibody	Cayman Chemical, Ann Arbor, MI, USA (Synthetic peptide from the human COX-2 sequence, amino acid 580-599, used as antigen, Catalog No. 160112)
Protease inhibitor cocktail	Sigma Chemical Co., St. Louis, MO, USA
Protein molecular weight marker	Amersham International plc, Little Chalfont, Buckinghamshire, UK
Skimmed milk	Snow Brand Inc., Victoria, Australia
Sodium chloride	Merck, Darmstadt, Germany
Sodium dodecyl sulfate	Sigma Chemical Co., St. Louis, MO, USA
TEMED	Bio Basic Inc., Toronto, Canada
Tris (hydroxymethyl) aminomethane	Research Organics Inc., St. Cleveland, OH, USA
Tween 20	Bio Basic Inc., Toronto, Canada
96-well plate	Nalge Numc International, Roskilde, Denmark

## APPENDIX B

### List of the instruments used in this study.

Instrument-Model	Source
Adjustable automatic pipette, LABMATE p10, p20, p200	High Tech Lab, Poland
SOCOREX p1,000	Swiss Made, Switzerland
Bench-top homogenizer, Con-Totque	Eberbach Corporation, USA
Densitometer, Model 1371	Helena Laboratories, USA
Electrophoresis and Electrotransfer Unit, V10-CDC and V10-EBGRM	Scie-Plas Limited, UK
Electronic balance, Libror EB-33OH	Shimadzu Corporation, Japan
Heating block, DB-101	General Enterprises Marketing, Thailand
Magnetic stirrer, Pyro-Magnestir	LAB-LINE, USA
Microplate reader, EL 340	Bio-TEK Instruments, USA
Multichannel motorized pipette, EDP-Plus M8	Rainin Instrument Company, USA
pH meter, Cyberscan 510	Eutech Instruments, Singapore
Power supply, ESP 500/400	Pharmacia Fine Chemicals, Sweden
Refrigerated centrifuge, 5417R	Eppendorf, Germany
Refrigerator (-80 °C), Ultra cold	P. T. W., Thailand
Shaker, VRN-200	Gemmy Industrial Corporation, Germany
Vortex mixer, VM-300	Gemmy Industrial Corporation, Germany
Water bath, WB 22	Memmert, Germany
X-ray film cassettes, PL-B 8x10 Inch	Okomoto Limited, Japan

## APPENDIX C

### Reagents and buffers preparation

#### 1. Reagents and buffers for SDS-PAGE

##### 1.1 Acrylamide solution

Acrylamide (FW 71.08)	60	g
Bis-acrylamide (FW 154.17)	1.6	g

Dissolved and made up to 200 ml with ddH<sub>2</sub>O.

Stored up to 3 months at 4 °C in the dark.

(Caution: Acrylamide is a neurotoxin and should be handle with care.)

##### 1.2 Running gel buffer (1.5M Tris-HCL, pH 8.8)

Tris (FW 121.1)	36.3	g
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Dissolved in 150 ml of ddH<sub>2</sub>O to pH 8.8 with concentration HCL and made up to 200 ml with ddH<sub>2</sub>O. Stored up to 3 months at 4 °C.

##### 1.3 Stacking gel buffer (0.5M Tris-HCl, pH 6.8)

Tris (FW 121.1)	3.0	g
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Dissolved in 40 ml of ddH<sub>2</sub>O to pH 6.8 with concentration HCL and made up to 50 ml with ddH<sub>2</sub>O. Stored up to 3 months at 4 °C.

##### 1.4 10% Ammonium persulfate (APS)

Ammonium persulfate (FW 228.2)	0.1	g
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Dissolved in 1 ml of ddH<sub>2</sub>O. Freshly prepared.

## 1.5 10% Sodium dodecyl sulfate (SDS)

SDS (FW 288.4)	0.1	g
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Dissolved and made up to 100 ml with ddH<sub>2</sub>O.

## 1.6 0.05% Bromphenol blue

Bromphenol blue (FW 669.99)	0.005	g
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Dissolved in 10 ml of dH<sub>2</sub>O. Stored at 4 °C.

## 1.7 SDS lysis buffer

SDS (FW 288.4)	2.0	g
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Glycerol (FW 92.09)	10	ml
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0.5M Tris-HCl, pH 6.8	12.5	ml
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Dissolved and made up to 100 ml with ddH<sub>2</sub>O.

## 1.8 Running buffer

Tris (FW 121.1)	15.15	g
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Glycine (FW 75.07)	72.05	g
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SDS (FW 288.4)	5.0	g
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Dissolved and made up to 5 l with dH<sub>2</sub>O.

## 2. Polyacrylamide gel preparation (two vertical electrophoresis gels, 8x10x0.75 cm)

following Laemmli's system

## 2.1 Running gel

Acrylamide solution	5	ml
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Running gel buffer	3.75	ml
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10% SDS	150	μl
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ddH <sub>2</sub> O	6	ml
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TEMED	10	ml
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APS	75	μl
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Swirled gently to mix and poured the solution into the gel cassette.

## 2.2 4% Stacking gel

Acrylamide solution	1.33	ml
Stacking gel buffer	2.5	ml
10% SDS	100	$\mu$ l
ddH <sub>2</sub> O	6	ml
TEMED	10	ml
APS	50	$\mu$ l

Swirled gently to mix and filled the top of the cassette with this mixture.

## 3. Reagents and buffers for Western blot analysis

## 3.1 Transfer buffer

Tris (FW 121.1)	15.15	g
Glycine (FW 75.07)	70.70	g

Dissolved in 1 l of methanol and made up to 5 l with ddH<sub>2</sub>O.

## 3.2 Tris buffer saline (TBS)-Tween, pH 7.5 (10X)

Tris (FW 121.1)	60	g
NaCl (FW 58.45)	90	g

Dissolved in 800 ml of dH<sub>2</sub>O to pH 7.5 with concentrate HCl and made up to 1 l with dH<sub>2</sub>O. Added 5 ml of Tween 20 and mix well.

## 3.3 5% BSA in TBS-Tween (Blocking buffer)

BSA (MW 66)	5	g
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Dissolved and made up to 100 ml with TBS-Tween, pH 7.5. Stored at -20 °C.

## 3.4 5% Skimmed milk in TBS-Tween

Skimmed milk	5	g
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Dissolved and made up to 100 ml with TBS-Tween, pH 7.5. Stored at -20 °C.

### 3.5 Stripping buffer, pH 6.7

(1) Tris (FW 121.1) 3.78 g

Dissolved in 400 ml of dH<sub>2</sub>O to pH 6.7 with concentrate HCl and made up to 500 ml with dH<sub>2</sub>O

(2) 2-mercaptoethanol 3.99 ml

SDS (FW 288.4) 10 g

Dissolved and made up to 500 ml with (1)

### 3.6 Developing solution

Stock developer 100 ml

dH<sub>2</sub>O 400 ml

Mix thoroughly and stored at RT.

### 3.7 Fixing solution

Stock Fixer 100 ml

dH<sub>2</sub>O 400 ml

Mix thoroughly and stored at RT.



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