



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
Autoadiographic 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
di-Sodium hydrogen phosphate	Merck, Darmstadt, Germany
ECL Western Blotting detection kit	Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK
Film developer	Eastman Kodak Company, Rochester, NY, USA
Film 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
Goat anti-rabbit IgG conjugated HRP	Dako, Glostrup, Denmark
Goat anti-VEGF polyclonal antibody	R&D System., Minneapolis, MN, USA (Catalog No. AF-293-NA)
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-VEGF monoclonal antibody	R&D System., Minneapolis, MN, USA (Catalog No. MAB293)
Mouse anti-GAPDH monoclonal antibody	Abcam Limited., Cambridgeshire, UK (Catalog No. ab8245)
Polyvinylidene fluoride	Pall corporation, UK
Potassium chloride	Merck, Darmstadt, Germany
Potassium dihydrogen phosphate	Merck, Darmstadt, Germany
Protease inhibitor cocktail	Sigma Chemical Co., St. Louis, MO, USA
Protein molecular weight marker	Fermentas Life Sciences., Hanover, MD, USA
Rabbit anti-VEGF polyclonal antibody	Santa Cruz Biotechnology Inc., California, USA)
Skimmed milk	Mission Brand Inc., Bangkok, Thailand
Sodium chloride	Merck, Darmstadt, Germany
Sodium dodecyl sulfate	Sigma Chemical Co., St. Louis, MO, USA
Sulfuric acid	Merck, Darmstadt, Germany
TEMED	Bio Basic Inc., Toronto, Canada
TMB	Zymed Laboratories Inc., San Francisco, USA
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 automatic pipette, MULTIMATE	High Tech Lab, Poland
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 ddH₂O.

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 ddH₂O and adjusted to pH 8.8 with concentrated HCL and made up to 200 ml with ddH₂O. 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 ddH₂O to pH 6.8 with concentration HCL and made up to 50 ml with ddH₂O. 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 ddH₂O. 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 ddH₂O.

1.6 0.05% Bromphenol blue

Bromphenol blue (FW 669.99)	0.005	g
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Dissolved in 10 ml of dH₂O. 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 ddH₂O.

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 dH₂O.

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

following Laemmli's system

2.1 10% 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 ₂ 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 15% Running gel

Acrylamide solution	7.5	ml
Running gel buffer	3.75	ml
10% SDS	150	μl
ddH ₂ O	3.55	ml
TEMED	10	ml
APS	75	μl

Swirled gently to mix and poured the solution into the gel cassette.

2.3 4% Stacking gel

Acrylamide solution	1.33	ml
Stacking gel buffer	2.5	ml
10% SDS	100	μl
ddH ₂ O	6	ml
TEMED	10	ml
APS	50	μl

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 ddH₂O.

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 dH₂O to pH 7.5 with concentrate HCl and made up to 1 l with dH₂O. Added 5 ml of Tween 20 and mix well.

3.3 2.5% Skimmed milk in TBS-Tween

Skimmed milk	2.5	g
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Dissolved and made up to 100 ml with TBS-Tween, pH 7.5.

3.4 Stripping buffer, pH 6.7

(1) Tris (FW 121.1)	3.78	g
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Dissolved in 400 ml of dH₂O to pH 6.7 with concentrate HCl and made up to 500 ml with dH₂O

(2) 2-mercaptoethanol	3.99	ml
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SDS (FW 288.4)	10	g
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Dissolved and made up to 500 ml with (1)

3.5 Developing solution

Stock developer	100	ml
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dH ₂ O	400	ml
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Mix thoroughly and stored at RT.

3.6 Fixing solution

Stock Fixer	100	ml
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dH ₂ O	400	ml
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Mix thoroughly and stored at RT.

4. Coomassie Blue staining solution and destain solution

4.1 Coomassie Blue staining solution

Coomassie Brilliant Blue R250	0.125	g
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Methanol	200	ml
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Acetic acid	35	ml
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Dissolved and made up to 500 ml with dH₂O.

Store at RT for up to 6 months

4.2 Destain solution I

Methanol	200	ml
Acetic acid	35	ml

Dissolved and made up to 500 ml with dH₂O.

Store at RT

4.3 Destain solution II

Methanol	50	ml
Acetic acid	70	ml

Dissolved and made up to 1 l with dH₂O.

Store at RT

5. Reagents and buffers for ELISA analysis

5.1 Phosphate buffer saline (PBS)(10X)

NaCl	40	g
KH ₂ PO ₄	1	g
Na ₂ HPO ₄	5.75	g
KCl	1	g

Dissolved reagent in dH₂O and made up to 1 l, store at RT

5.2 Phosphate buffer saline (PBS)(1X)

Stock PBS 10X	100	ml
dH ₂ O	900	ml

Mix thoroughly and stored at RT.

5.3 Phosphate buffer saline-Tween (PBS-T)

Stock PBS 10X	100	ml
dH ₂ O	900	ml
Tween 20	500	μl

Made up volume to 1 l, stored at RT

5.4 5% BSA in PBS

BSA	2.5	g
PBS 1X	50	ml
Stored at -20°C		

5.5 1.5% Skimmed milk (blocking solution)

Skimmed milk (Mission)	1.5	g
PBS 1X	100	ml

5.6 1M H_2SO_4 (stop solution)

18.76 M H_2SO_4	11	ml
dH_2O	189	ml

(dispense H_2SO_4 in to water)

The solution was carefully mixed and stored at RT.

CURRICULUM VITAE

Name

Miss.Onusa Wattananupong

Date of Birth

October 22, 1978

Education

March 2000

Bachelor of Science in Medical Technology,
B.Sc. (Med. Tech.)
Faculty of Medical Technology,
Huachiew Chalermprakiet University

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