APPENDIX

APPENDIX A

List of the reagents and chemicals used in this study as following:

Name of chemical/ reagent	Source/ Company
Absolute ethanol	Merck, Darmstadt, Germany
Acrylamide	Bio-Rad, Richmond, CA, USA
Ammonium persulfate (APS)	Bio-Rad, Richmond, CA, USA
Anti-mouse cyclin A IgG	BD Biosciences™ San Jose, CA,USA
Anti-mouse cyclin B IgG	BD Biosciences™ San Jose, CA,USA
Anti-mouse cyclin ER IgG	EMD Millipore, Darmstadt, Germany
Anti-mouse cdc2	BD Biosciences™ San Jose, CA,USA
Anti-mouse p53 IgG	BD Biosciences™ San Jose, CA,USA
Anti-rabbit GAPDH polyclonal antibody	Santa Cruz Biotechnology, Lake Placid, NY, USA
Anti-rabbit IgG HRP conjugate	Promega, Madison WI, USA
Bovine serum albumin	PIERCE, Rockford, IL, USA
Bromphenol blue	Sigma-Aldrich, St. Louis, MO, USA
Copper sulfate	Merck, Darmstadt, Germany
Developer and replenisher	Kodak, NY, USA
Diethyl sulfoxide (DMSO)	Sigma-Aldrich, St. Louis, MO, USA LAB-SCAN, Bangkok, thailand
Disodium hydrogen phosphate	Merck, Darmstadt, Germany Fluka, Buchs, Switzerland
Fetal bovine serum	GIBCO-BRL, Grand Island, NY, USA
Folin & Cocalteu's phenol reagent	Merck, Darmstadt, Germany
Glycerol	Merck, Darmstadt, Germany
Glycine	Amresco, Ohio, USA
HEPES	Sigma-Aldrich, St. Louis, MO, USA
Hydrochloric acid (HCL)	Merck, Darmstadt, Germany

Isopropanol	Merck, Darmstadt, Germany
L-glutamine	Invitrogen™, Carlsbad, CA, USA
Magnesiumchloride	Merck, Darmstadt, Germany
Mercaptoethanol	Sigma-Aldrich, St. Louis, MO, USA
Methanol	LAB-SCAN, Bangkok, Thailand
MTT	Sigma-Aldrich, St. Louis, MO, USA
PageBlue™ Protein Staining Solution	Fermentas, Maryland , USA
PageRuler™ Prestained Protein Ladder	Fermentas, Maryland , USA
Penicillin-streptomycin	Invitrogen™, Carlsbad, CA, USA
Potassium chloride	Merck, Darmstadt, Germany
Potassium dihydrogen phosphate	Merck, Darmstadt, Germany
Propidium iodide	Sigma-Aldrich, St. Louis, MO, USA
Restore™ Western Blot Stripping Buffer	PIERCE, Rockford, IL, USA
RNase A	Invitrogen™, Carlsbad, CA, USA
RPMI-1640 powder	Invitrogen™, Carlsbad, CA, USA
Sheath Fuild IsoFlower	IsoFlow™ , Backman coulter, USA
Skim milk	Nestle, Thailand
Sodium bicarbonate	Merck, Darmstadt, Germany
Sodium carbonate	Merck, Darmstadt, Germany
Sodium chloride	Merck, Darmstadt, Germany
Sodium dodecyl sulfate (SDS)	Sigma-Aldrich, St. Louis, MO, USA
Sodium potassium Tartrate	Sigma-Aldrich, St. Louis, MO, USA
SuperSignal® Chemiluminescent substrate West Pico	PIERCE, Rockford, IL, USA
TEMED	Bio-Rad, Richmond, CA, USA
Trypan blue	AMRESCO®, Solon, Ohio
Tween 20	Sigma-Aldrich, St. Louis, MO, USA
Water, sterile, nuclease-free	Amresco, Ohio, USA

APPENDIX B

List of the instrument used in this study as following:

Instrument	Company
Analytical balance	Mettler Toledo, Küsanacht, Switzerland
Autoclave	Tomy, Seiko, Tokyo, Japan
Automatic pipette	Biohit, Finland and Bio-rad, USA
Automatic pipette tip	Bioline, UK
Carbon dioxide incubator	Shel Lab, OR, USA
Centrifuge	MPW med instruments, Warsaw, Poland
Centrifuge tube (15 and 50 mL)	SPL life Sciences, Korea
Deionized distilled water machine	PK water text
Medical X-ray film	AGFA HEALTHCARE, China
Freezer (-80^{o}C)	PTW ultra cold, China
Freezer (-20^{o}C)	Sanyo, Japan
Flowcytometer	Cytomics FC 500, Beckman Coulter,USA
FlowJo V10 program	FlowJo, LLC data analysis, USA
Gel documentation	Bio-Rad, USA
10 cm glass plate	PYREX, USA; and PETRIO
Hotplate	Daihan Labtech LLC., DE, USA
Inverted microscope	Olympus, Japan
Laminar flow biological carbinet	Clean, Tamil Nadu, India
Light microscope	Olympus, Japan
Microcentrifuge	Eppendrof, Germany
Microplate reader	Metertech, Taipei, Taiwan
PVDF membrane	Millipore, Darmstadt, Germany
Pipette-aid	Drummond, USA

Pasture pipette	Pyrex, USA
pH meter	Thermo Orion, USA
Quantity One version 4.6.3	Bio-Rad Laboratories, Hercules, CA, USA
Sonicator bath	BIORUPTOR®, USA
Power supply	E-C apparatus corporation, USA
Refrigerator	Toshiba, Japan
Serological pipette	Pyrex, USA
Spectrophotometer	Shimadzu, Japan
25 or 75 cm^3 T-flask	NUNC, Jiangsu, China
Trans-blot® electrophoretic transfer cell	Bio-Rad, Richmond, CA, USA
Vortex mixer	Gemmy industrial corporation
Water bath	Witeg, Korea

APPENDIX C

Methods of preparation for the reagents used

1. Reagents for leukemic cell lines culture

1.1) Incomplete RPMI 1640 medium

RPMI-1640 power medium (1 pack)	10.4	g
HEPES	3.57	g
$NaHCO_3$	2.0	g
0.34% 2-Mercaptoethanol	1.0	mL
Deionized distilled water up to 1,000 mL		

Medium was sterilized by filtration through suction filter with 0.2 µm filter membrane. Then the sterility was checked before use, and stored at 4ºC.

1.2) Complete RPMI-1640 medium

Incomplete RPMI-1640 medium	88.5	mL
FBS	10.0	mL
100 units/mL penicillin and 100 µg/mL streptomycin	1.0	mL
200 mM L-glutamine	0.5	mL

Medium was checked for sterility before use, and stored at 4ºC.

1.3) Freezing solution

FBS	9.2	mL
DMSO	0.8	mL

1.4) Phosphate buffer saline (PBS), pH 7.4

KH_2PO_4	0.24	g
Na_2HPO_4	1.44	g
NaCl	8.0	g
KCl	0.2	g

All substances were dissolved in 800 mL of deionized distilled water and adjusted to pH 7.4, then top up to 1,000 mL and sterilized by autoclave.

2. Reagents for cell survival measurement

MTT stock dye solution

MTT	1.0	g
PBS, pH 7.4	200	mL

After dissolve MTT dye, filtrate any nonsoluble powder by filtration with 0.2 μm filter membrane, collected in dark container.

3. Reagents for protein determination (Folin-Lowry method)

3.1) Reagent A

2% (w/v) Na_2CO_3 in 0.1 N NaOH

NaOH	2.0	g
Na_2CO_3	10	g
Deionized distilled water	500	mL

3.2) Reagent B

0.5% (w/v) $CuSO_4 \cdot 5H_2O$ and 1% (w/v) $NaKC_4H_4O_6 \cdot 2H_2O$ (Na-K Tartrate) Two reagents, $CuSO_4$ and Na-K Tartrate, were prepared as follow:

Part A: 0.5% (w/v) $CuSO_4 \cdot 5H_2O$

$CuSO_4 \cdot 5H_2O$	0.5	g
Deionized distilled water	50	mL

Part B: 1% (w/v) Na-K Tartrate

Na-K Tartrate	1.0	g
Deionized distilled water	50	mL

Before using 0.5 mL of part A and part B were mixed.

3.3) Reagent C

Working solution was prepared by mixing reagent A 50 mL and reagent B ratio 50:1.

3.4) Folin-ciocalteau phenol reagent 1 N

Folin-ciocalteau phenol reagent 2 N was diluted in DI water to 1 N.

4. Reagents for SDS-PAGE and Western blotting

4.1) Separating gel buffer 1.5 mM Tris-HCl, pH 8.8

Tris-base	18.15	g

Deionized distilled water	80	mL

Adjust pH to 8.8 then adjust volume to 100 mL and filtrate any nonsoluble powder by filtration with membrane filter pore size 0.2 µm.

4.2) 30% acrylamide solution

Acrylamide	29.2	g
Bis (Estaman)	0.8	g
Deionized distilled water	70	mL

Adjust volume to 100 mL and filtrate any nonsoluble powder by filtration with membrane filter pore size 0.2 µm, collect in dark container.

4.3) Stacking gel buffer 1.0 mM Tris-HCl, pH 6.8

Tris base	6.05	g
Deionized distilled water	70	mL

Adjust pH to 8.8 then adjust volume to 100 mL and filtrate any nonsoluble powder by filtration with membrane filter pore size 0.2 µm.

4.4) 10% Ammonium persulfate (APS) stock solution

APS	0.1	g
Deionized distilled water up to 1.0 mL		

4.5) 10% SDS stock solution

SDS	0.1	g
Deionized distilled water	1.0	mL

4.6) 7.5% Separating gel (1 gel)

Deionized distilled water	2.425	mL
1.5 mM Tris-HCl, pH 8.8	1.25	mL
10% SDS	50	µL
30% acrylamide solution	4.0	mL
10% APS	25	µL
TEMED	2.5	µL

4.7) 12% Separating gel (1 gel)

Deionized distilled water	3.5	mL
1.5 mM Tris-HCl, pH 8.8	2.5	mL
10% SDS	100	µL
30% acrylamide solution	4.0	mL
10% APS	50	µL
TEMED	5.0	µL

4.8) 4% Stacking gel buffer (1 gel)

Deionized distilled water	1.525	mL
1.0 mM Tris-HCl, pH 6.8	0.625	mL
10% SDS	25	µL
30% acrylamide solution	0.325	mL
10% APS	12.5	µL
TEMED	2.5	µL

4.9) 5X reducing buffer

5X non-reducing buffer	475	µL
2-Mercaptoethanol	25	µL

4.10) Electrode buffer (Running buffer)

Tris-base	3.0	g
Glycerol	14.4	g
SDS	1.0	g
Deionized distilled water up to 1,000 mL		

4.11) Transfer buffer (Blotting buffer)

Tris-base	3.0	g
Glycerol	14.4	g
Methanol	200	mL
Deionized distilled water up to 1,000 mL		

4.12) Washing buffer

PBS, pH 7.4	1,000	mL
Tween 20	1.0	mL

4.13) Phosphate buffer saline (PBS), pH 7.4

NaH_2PO_4	0.24	g
Na_2HPO_4	1.44	g
NaCl	8.0	g
Deionized distilled water up to 1,000 ml		

4.14) Blocking reagent

Skim milk	5.0	g
PBS, pH 7.4	100	mL

5. Reagent for cell cycle

5.1) Propidium Iodide

Propidium Iodide	1.0	mg
Distilled water	1.0	mL

PI stock Solution (1 mg/mL) Weigh out PI and place in 15 mL conical tube. Dilute PI to 1 mg/mL with distilled water. Cover tube with foil and store refrigerated.

CURRICULUM VITAE

Name	Miss Wipavee Uthawang
Date of birth	December 13, 1989
Place of birth	Phayao, Thailand
Education	2007, Certificate of senior high school from Srinagarindra the Princess Mother School, Phayao, Thailand 2011, Bachelor of Science (Medical Technology), Faculty of Medical Technology, Phayao University, Phayao, Thailand.