APPENDIX

APPENDIX A

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

Chemical name	Source
Absolute ethanol	Merck, Darmstadt, Germany
Acrylamide	Bio-Rad, Richmond, CA, USA
Ammonium persulfate (APS)	Bio-Rad, Richmond, CA, USA
Anti-rabbit FLT3 extracellular domain	Invitrogen^{TM}, Carlsbad, CA, USA
Anti-rabbit Bcr/Abl	USBiological life sciences, USA
Anti-rabbit WT1	Santa Cruz Biotechnology, Lake Placid, NY, USA
Anti-rabbit GAPDH polyclonal antibody	Santa Cruz Biotechnology, Lake Placid, NY, USA
Anti-rabbit IgG HRP conjugate	Promega, Madison WI, USA
Bromphenol blue	Sigma-Aldrich, St. Louis, MO, USA
Bovine serum albumin (BSA)	PIERCE, Rockford, IL, USA
Disodium hydrogen sulfate	Merck, Darmstadt, Germany
DMSO	Sigma-Aldrich, St. Louis, MO, USA
Fetal bovine serum (FBS)	Invitrogen^{TM}, Carlsbad, CA, USA
Folin-Ciocalteu's phenol	Merck, Darmstadt, Germany
Glycerol	Merck, Darmstadt, Germany
Luminata^{TM} Forte Western HRP Substrate	Millipore Corporation, Billerica, MA, USA

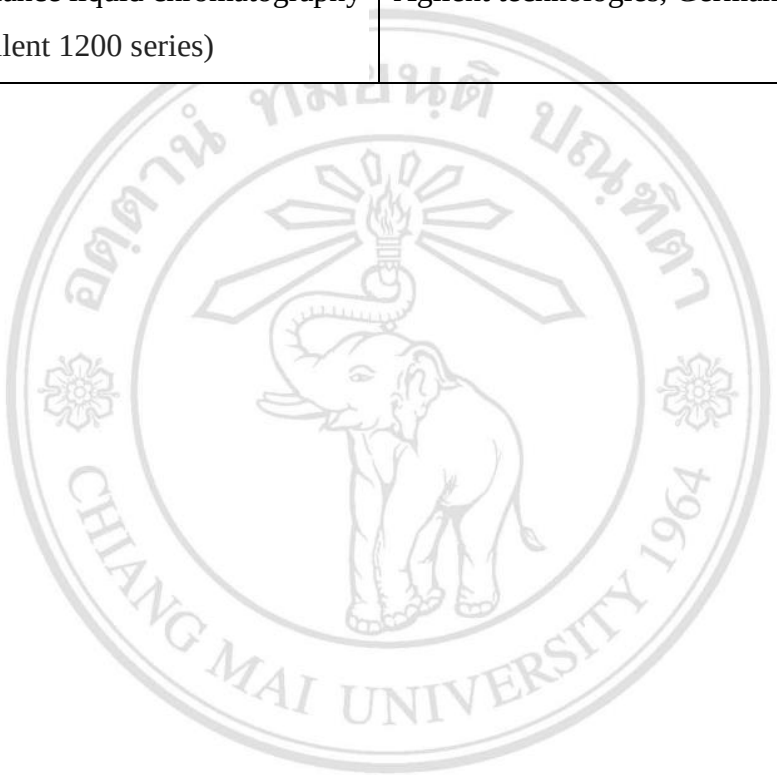
Chemical name	Source
L-glutamine	Invitrogen™, Carlsbad, CA, USA
Mercaptoethanol	GE healthcare, Uppsala, Sweden
Methanol	Merck, Darmstadt, Germany
Pageruler™ prestained protein ladder	Thermo Scientific, Rockford, IL, USA
Penicillin/streptomycin	Invitrogen™, Carlsbad, CA, USA
Potassium chloride	Merck, Darmstadt, Germany
Potassium dihydrogen phosphate	Merck, Darmstadt, Germany
Restore™ plus Western blot stripping	Thermo Scientific, Rockford, IL, USA
RPMI 1640	Invitrogen™, Carlsbad, CA, USA
SDS	Vivantis, Oceanside, CA, USA
Skim milk	Nestle, Thailand
Sodium bicarbonate	Merck, Darmstadt, Germany
Sodium carbonate	Merck, Darmstadt, Germany
Sodium chloride	Merck, Darmstadt, Germany
Sodium dihydrogen phosphate	Merck, Darmstadt, Germany
Sodium hydrogen carbonate	Merck, Darmstadt, Germany
Sodium potassium tartrate	Sigma-Aldrich, St. Louis, MO, USA
TEMED	Bio-Rad, Richmond, CA, USA
Tris	Vivantis, Oceanside, CA, USA
Trypan blue	AMRESCO®, Solon, Ohio
Tween 20	Sigma-Aldrich, St. Louis, MO, USA

APPENDIX B

List of the instrument used in this study as following:

Instruments	Source
Analytical balance	Mettler Toledo, Küsanacht, Switzerland
Autoclave	Tomy, Seiko, Tokyo, Japan
Automatic pipette	Biohit, Finland and Bio-rad, USA
Automatic pipette tip	Bioline, UK
Carbondioxide incubator	Shel Lab, OR, USA
Centrifuge	MPW med instruments, Warsaw, Poland
Centrifuge tube (15 and 50 ml)	SPL life Sciences, Korea
Medical X-ray film	AGFA HEALTHCARE, China
Hotplate	Daihan Labtech LLC., DE, USA
Laminar flow biological carbinet	Clean, Tamil Nadu, India
Light microscope	Olypus, Germany
Microcentrifuge	Eppendrof, Germany
Microplate reader	Metertech, Taipei, Taiwan
pH meter	E-Z-DO Company, NJ, USA
Power supply	E-C apparatus corporation, USA
PVDF membrane	Millipore, Darmstadt, Germany
Quantity One Version 4.6.3	Bio-rad Laboratories, Hercules, CA, USA
Sonicator bath	BIORUPTOR®, USA

Instruments	Source
T-flask (25 cm^3)	NUNC, Jiangsu, China
Trans-blot® electrophoresis transfer set	Bio-rad, Richmond, CA, USA
Vortex mixer	Gemmy industrial coaporation, Taiwan
Water bath	Daihan sciencific, Korea
High performance liquid chromatography analyzer (Agilent 1200 series)	Agilent technologies, Germany



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

2.1 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

CURRICULUM VITAE

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