APPENDIX A

Chemicals

Chemical name	Source
Absolute ethanol	Merck, Darmstadt, Germany
Acrylamide	Bio-Rad, Richmond, CA, USA
Albumin bovine fraction V	Bio-Rad, Richmond, CA, USA
Ammonium persulfate (APS)	Bio-Rad, Richmond, CA, USA
Anti-FLT3 monoclonal antibody R-PE	Invitrogen™ , Carlsbad, CA, USA
Anti-mouse FLT3 extracellular domain	Upstate Biotechnology, Lake Placid, NY, USA
Anti- rabbit GAPDH polyclonal antibody	Santa Cruz Biotechnology, Santa Cruz, CA, USA
Anti-rabbit IgG HRP conjugate	Promega, Madision, WI, USA
Bis	Bio-Rad, Richmond, CA, USA
Bromphenol blue	Sigma-Aldrich, St. Louis, MO, USA
BSA	PIERCE, Rockford, IL, USA
Coulter Isoton II diluent	Beckman Coulter, Brea, CA, USA
Disodium hydrogen phosphate	Merck, Darmstadt, Germany
DMSO	Sigma-Aldrich, St. Louis, MO, USA
DNA-Dye NonTox	AppliChem, Darmstadt, Germany
EDTA	Merck, Darmstadt, Germany
Fetal bovine serum (FBS)	Invitrogen™ , Carlsbad, CA, USA
Folin-Ciocalteu's phenol	Merck, Darmstadt, Germany
GeneRuler 1 kb DNA Ladder	Thermo Scientific, Rockford, IL, USA
Glycerol	Merck, Darmstadt, Germany

Chemical name	Source
IMDM medium	Invitrogen™ , Carlsbad, CA, USA
L-glutamine	Invitrogen™ , Carlsbad, CA, USA
Luminata™ Forte Western HRP Substrate	Millipore Corporation, Billerica, MA, 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
Primers	Invitrogen™ , Carlsbad, CA, USA
Restore™ plus Western blot stripping	Thermo Scientific, Rockford, IL, USA
RIPA buffer	Thermo Scientific, Rockford, IL, USA
RNase-free water	Invitrogen™ , Carlsbad, CA, USA
RNaseOUT™ Recombinant Ribonu-clease Inhibitor	Invitrogen™ , Carlsbad, CA, USA
RPMI-1640	Invitrogen™ , Carlsbad, CA, USA
SDS	Vivantis, Oceanside, CA, USA
Skim milk	Fluka, Buchs, Switzerland
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
SuperScript™ III one-step RT-PCR System with Platinum® *Taq* DNA Polymerase	Invitrogen™ , Carlsbad, CA, USA
TEMED	Bio-Rad, Richmond, CA, USA

Chemical name	Source
Tris	Vivantis, Oceanside, CA, USA
TRIZOL Reagent®	Invitrogen™ , Carlsbad, CA, USA
Trypan blue	Sigma-Aldrich, St. Louis, MO, USA
Tween 20	Sigma-Aldrich, St. Louis, MO, USA

APPENDIX B

Instruments

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
Carbon dioxide incubator	Shel Lab, OR, USA
CELLQUEST™ software	Becton Dickinson, NJ, USA
Centrifuge	MPW med instruments, Warsaw, Poland
Centrifuge tube (15 and 50 ml)	SPL life Sciences, Korea
CL-XPosure ™ film	Thermo Scientific, Rockford, IL, USA
Gel Electrophoresis system EG-100	BIOER TECHNOLOGY CO.LTD.,
Flow cytometer	Becton Dickinson, NJ, USA
Hotplate	Daihan Labtech LLC., DE, USA
Laminar flow biological cabinet	Clean, Tamil Nadu, India
Light microscope	Olympus, Japan
Mastercycler® personal	Eppendorf, Germany
Microcentrifuge	Eppendorf, Germany
Microplate reader	Metertech, Taipei, Taiwan
pH meter	E-Z-Do Company, NJ, USA
Power supply	E-C apparatus corporation, USA
PVDF membrane	BIO-RAD LABORATORIES, Hercules, CA, USA

Instruments	Source
Quantity One *Version 4.6.3*	BIO-RAD LABORATORIES, Hercules, CA, USA
Sonicator bath	GFL, Burgwedel, Germany
Spectrophotometer	Shimadzu, Japan
T-flask (25 and 75 cm^3)	SPL life Sciences, Korea
Trans-blot® electrophoresis transfer set	Bio-Rad, Richmond, CA, USA
Vortex mixer	Gemmy industrial corporation, Taiwan
Water bath	Daihan scientific, Korea

APPENDIX C

Reagents Preparation

1. Reagents for leukemic cell lines culture

1.1 Incomplete RPMI-1640 medium

RPMI-1640 power medium	10.4	g (1 pack)
HEPES	3.57	g
$NaHCO_3$	2.0	g
0.34% 2-Mercaptoethanol	1.0	ml
DI water q.s. 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 4°C.

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 4°C.

1.3 Incomplete IMDM medium

IMDM medium	1	g (1 pack)
HEPES	3.57	g
$NaHCO_3$	2.0	g
0.34% 2-Mercaptoethanol	1.0	ml
DI water q.s. 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 4°C.

1.4 Complete IMDM medium

Incomplete IMDM 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 4°C.

1.5 Freezing solution

FBS	9.2	ml
DMSO	0.8	ml

1.6 Phosphate buffer saline (PBS), pH 7.4

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

DI water q.s. to 1,000 ml

All substances were dissolved in 800 ml of DI water and adjusted to pH 7.2. After that volume was adjusted to 1,000 ml and sterilized in an autoclave.

1.7 0.2% Trypan blue

Trypan blue	0.2	g
PBS	100	ml

2. Reagents for flow cytometry

2.1 FACS diluents (1% BSA-0.02% NaN_3 in PBS)

BSA fraction V	10.0	g
NaN_3	0.2	g

PBS, pH 7.2 q.s. to 1,000 ml

2.2 1% Paraformaldehyde

Paraformaldehyde	5.0	g
PBS, pH 7.2 q.s. to 500 ml		

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

3.1 Reagent A (2% Na_2CO_3 in 0.1 N NaOH)

NaOH	2.0	g
Na_2CO_3	10.0	g
DI water q.s. to 500 ml		

3.2 Reagent B (0.5% $CuSO_4$ 5 H_20 in 1% $NaKC_4H_4O_62$ H_2O (Na-K Tartrate))

0.5% $CuSO_4$•$5H_2O$

$CuSO_4$ 5 H_2O	0.5	g
DI water q.s. to 50 ml		

1% $NaKC_4H_4O_6$•$2H_2O$ (Na-K Tartrate)

Na-K Tartrate	1.0	g
DI water q.s. to 50 ml		

Reagent B was prepared by mixing $CuSO_4$ and Na-K Tartrate ratio 1:1.

3.3 Reagent C

Reagent C was freshly prepared by mixing Reagent A and B ratio 50:1.

3.4 Folin-ciocalteau phenol reagent 1 N

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

3.5 Bovine serum albumin (BSA) stock solution

BSA	0.01	g
DI water	10	ml

4. Reagents for SDS-PAGE and Western blotting

4.1 1.5 mM Tris-HCl, pH 8.8

Tris-base	18.15	g
DI water q.s. to 100 ml		

4.2 30% acrylamide solution

Acrylamide	29.2	g
Bis	0.8	g
DI water q.s. to 100 ml		

4.3 1.0 mM Tris-HCl, pH 6.8

Tris-base	6.05	g
DI water q.s. to 100 ml		

The pH was adjusted to 6.8 and the volume was adjusted to 100 ml.

4.4 10% Ammonium persulfate (APS) stock solution

APS	0.1	g
DI water q.s. to 1.0 ml		

4.5 10% SDS stock solution

SDS	0.2	ml
DI water q.s. to 1.0 ml		

4.6 7.5% Separating gel (1 gel)

DI 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 4% Stacking gel buffer (1 gel)

DI 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

4.8 5X reducing buffer

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

4.9 Electrode buffer (Running buffer)

Tris-base	3.0	g
Glycerol	14.4	g
SDS	1.0	g
DI water q.s. to 1,000 ml		

4.10 Transfer buffer (Blotting buffer)

Tris-base	3.0	g
Glycerol	14.4	g
Methanol	200	ml
DI water q.s. to 1,000 ml		

All substances were dissolved and adjusted to 1,000 ml with DI water.

4.11 Washing buffer

PBS, pH 7.4	1,000	ml
Tween 20	1	ml

4.12 Phosphate buffer saline (PBS), pH 7.4

NaH_2PO_4	0.204	g
Na_2HPO_4	1.3	g
NaCl	7.28	g
DI water q.s. to 1,000 ml		

All substances were dissolved in DI water and adjusted to pH 7.4.

4.13 Blocking reagent

Skim milk	5.0	g
PBS, pH 7.4	100	ml

5. Reagents for RT-PCR

5.1 DEPC treated water

DI water	1,000	ml
DEPC	100	µl

DEPC treated water was shaken and stored at room temperature overnight and DEPC removed by autoclaving.

5.1 50X TAE (Tris-acetate-EDTA) stock

Tris-base	242	g
Glacial acetic acid	57.1	ml
0.5 M EDTA	100	ml

DI water q.s. to 1,000 ml

To make 1x TAE, 20 ml of 50X TAE stock dilute into 980 ml of DI water.

5.2 1% w/v Agarose gel

Agarose	1.0	g

1X TAE q.s. to 100 ml

5.3 Hypotonic solution (0.083% NH_4Cl) for RBC lysing

NH_4Cl	0.829	g
$KHCO_3$	0.1	g
EDTA	3.7	mg

DI water q.s. to 100 ml

All substances were dissolved and adjusted to pH 7.2 with 1 N HCl.

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