APPENDICES

Appendix A

The following reagents and chemicals used in this study

Name of reagent, commercial kit or chemical substance	Company
Commercial kits	
Human Phospho-Kinase Array Kit	R&D systems, USA
Chemical substances	
Absolute ethanol (C_2H_5OH)	Merck, Germany
Absolute methanol (CH_3OH)	Merck, Germany
Acetic acid (CH_3COOH)	Merck, Germany
Acrylamide (C_3H_5NO)	Bio-Rad, USA
Agarose	Sigma-Aldrich, USA
Ammonium persulfate (APS; $(NH_4)_2S_2O_8$)	BioRad, USA
Bisacrylamide (N,N'-Methylene-bis-acrylamide; $C_2H_5NO_2$)	BioRad, USA
Bovine serum albumin (BSA)	Sigma-Aldrich, USA
Bromphenol blue ($C_{19}H_{10}Br_4O_5S$)	Sigma-Aldrich, USA
Calcium chloride ($CaCl_2$)	Merck, Germany
Coomasie brilliant blue R-250 ($C_{45}H_{44}N_3NaO_7S_2$)	Sigma-Aldrich, USA
Copper sulfate ($CuSO_4$)	Merck, Germany
Developer and replenisher	Kodak

Diethyl pyrocarbonate (DEPC; $C_6H_{10}O_5$)	Sigma-Aldrich, USA
Dimetyl sulfoxide (DMSO; $(CH_3)_2SO$)	Sigma-Aldrich, USA
Disodium hydrogen phosphate (Na_2HPO_4)	Merck, Germany
DNA lader - 1 kb DNA Ladder - O'GeneRuler™ 1 kb DNA Ladder - O'GeneRuler™ 100 bp DNA Ladder	 Invitrogen, USA MBI, Fermentas, Canada MBI, Fermentas, Canada
Ethidium bromide ($C_{21}H_{20}BrN_3$)	Sigma-Aldrich
Ethylenediaminetetraacetic acid dipotassium dihydrate; EDTA ($C_{10}H_{14}K_2H_2O_8 \cdot 2H_2O$)	Fluka, USA
Deoxycholic acid ($C_{24}H_{40}O_4$)	Sigma-Aldrich, USA
Disodium dihydrogen ethylenediaminetetraacetate; disodium salt EDTA ($C_{10}H_{14}O_8N_2Na_2 \cdot 2H_2O$)	Fluka, USA
Fetal bovine serum	Invitrogen, USA
Folin & Clocalteu's phenol reagent	Merck, Germany
37% Formaldehyde (CH_2O)	Sigma-Aldrich, USA
Glacial acetic acid ($C_2H_4O_2CH_3COOH$)	Fluka, USA
Glycerol ($C_3H_5(OH)_3$)	Merck, Germany
Glycine ($C_2H_5NO_2$)	Fluka, USA
N-[2-hydroxyethy] piperazine-*N* [2-ethanesulfonic acid] (HEPES)	Sigma-Aldrich, USA
Hydrochloric acid (HCl)	Merck, Germany

Isopropanaol (C_3H_8O)	Merck, Germany
L-glutamine ($C_5H_{10}N_2O_3$)	Invitrogen, USA
LB-agar	Sigma-Aldrich, USA
LB-broth	Sigma-Aldrich, USA
Lithium chloride (LiCl)	Sigma-Aldrich, USA
2-Mercaptoethanol (C_2H_6OS)	Sigma-Aldrich, USA
MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]	Sigma-Aldrich, USA
Nonidet P 40 (Igepal CA-630)	Sigma-Aldrich, USA
Manganese(II) chloride ($MnCl_2{\cdot}4H_2O$)	Sigma-Aldrich, USA
PageBlue™ Prestained protein Ladder	MBI, Fermentas, Canada
Paraformaldehyde ($OH(CH_2O)_nH$ (n = 8 - 100)	Sigma-Aldrich, USA
Potassium chloride (KCl)	Merck, Germany
Potassium dihydrogen phosphate (KH_2PO_4)	Merck, Germany
RPMI-1640 powder	Invitrogen, USA
RPMI-1640 with L-glutamine medium	Invitrogen, USA
Purified bovine serum albumin (BSA)	New England Biolabs, USA
Skim milk (non fat milk)	Sigma-Aldrich, USA
Sarkosyl; Sodium lauroylsarcosinate; Sodium *N*-lauroylsarcosinate ($C_{15}H_{28}NNaO_3$)	Sigma-Aldrich, USA
Sodium azide (NaN_3)	Merck, Germany

Sodium bicarbonate ($NaCHO_3$)	Merck, Germany
Sodium chloride (NaCl)	Merck, Germany
Sodium dodecyl sulfate (SDS or NaDS) ($C_{12}H_{25}SO_4Na$)	Sigma-Aldrich, USA
Sodium dihydrogen phosphate dehydrate ($NaH_2PO_4 \cdot 2H_2O$)	Merck, Germany
Sodium potassium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$)	Sigma-Aldrich, USA
Sucrose ($C_{12}H_{22}O_{11}$)	Sigma-Aldrich, USA
Tris-base ($C_4H_{11}NO_3$)	Merck, Germany
2-[4-(2-sulfoethyl)piperazin-1-yl]ethanesulfonic acid; PIPES ($C_8H_{18}N_2O_6S_2$)	Sigma-Aldrich, USA
TEMED (*N, N, N′, N*′-tetramethylethylenediamine; $C_6H_{16}N_2$)	BioRad, USA
Trition X-100	Sigma-Aldrich, USA
Trypan blue ($C_{34}H_{28}N_6O_{14}S_4$)	Sigma-Aldrich, USA
Tween 20	Sigma-Aldrich, USA
Reagent kit *DC* protein Assay	BioRad, USA
Restore Western blot stripping buffer	Thermo Scientific, USA
SuperSignal® West Pico Chemiluminacent Substrate	Thermo Scientific, USA
SuperSignal® West Femto Chemiluminacent Substrate	Thermo Scientific, USA
QIAprep® Spin Miniprep Kit (Cat # 27106)	Qiagen, USA
QIAGEN® Plasmid Maxi Kit	Qiagen, USA

RNeasy® Mini Kit (Cat# 74104)	Qiagen, USA
QIAquick Gel Extraction Kit (Cat#28706)	Qiagen, USA
Dual-Luciferase® Reporter Assay System Chemistry	Promega, USA
β-galactosidase kit	Promega, USA
Lipofectamine™ LTX with Plus™ Reagent	Invitrogen, USA
Protease inhibitor AEBSF (4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride; $C_8H_{10}FNO_2S{\cdot}HCl$)	Calbiochem, USA
PMSF (phenylmethanesulfonylfluoride or phenylmethylsulfonyl fluoride; $C_7H_7FO_2S$)	Calbiochem, USA
Aprotinin (A-1153; $C_{284}H_{432}N_{84}O_{79}S_7$)	Sigma-Aldrich, USA
Leupeptin (L-2884)	Sigma-Aldrich, SA
DTT (Dithiothreitol; $C_4H_{10}O_2S_2$)	Sigma-Aldrich, USA
Kinase phosphorylation inhibitor PKC inhibitor (GF109203x; $C_{25}H_{24}N_4O_2$)	TOCRIS, England
PKC inhibitor (GÖ6976; $C_{24}H_{18}N_4O$)	TOCRIS, England
Jun N-terminal kinase inhibitor (SP600125; $C_{14}H_8N_2O$)	A.G. Scientific, USA
PI3K inhibitor (LY294002; $C_{19}H_{17}NO_3$)	Promega, USA
MEK inhibitor (U0126; $C_{18}H_{16}N_6S_2$)	Promega, USA
Drug Pure curcumin	In house
Protein synthesis inhibitor Cycloheximide ($C_{15}H_{23}NO_4$)	Calbiochem, USA

Proteasome inhibitor (-)-Epigallocatechin gallate (EGCG; $C_{22}H_{18}O_{11}$)	Sigma-Aldrich, USA
MG 132 ($C_{26}H_{41}N_3O_5$)	Calbiochem, USA
Lactacystin ($C_{15}H_{24}N_2O_7S$)	TRC, Ontario, Canada
Transcription inhibitor Actinomicine D ($C_{62}H_{86}N_{12}O_{16}$)	Sigma-Aldrich, USA
Antibiotic drug Geneticin (G418; $C_{20}H_{40}N_4O_{10}$)	Roche, USA
Ampicillin ($C_{16}H_{19}N_3O_4S$) (100 mg/mL)	Calbiochem, USA
Penicillin and Streptomycin solution (10,000 I.U/mL-Penicillin and 10,000 μg/mL-Streptomycin)	Gibco BRL Life Technologies, USA
Amino acid L-glutamine ($C_5H_{10}N_2O_3$) (200 mM)	Gibco BRL Life Technologies, USA
Plasmid vector Myr.PKCα.FLAG	Addgene, USA
PAD-Track CMV.GFP	Addgene, USA
pcDNA3.1.GFP	Sweeney's laboratory
pcDNA3.1 Lrig1.myc	Sweeney's laboratory
pcDNA3.1 p95 ErbB2	Sweeney's laboratory
pcDNA3.1 ErbB3	Sweeney's laboratory

pcDNA3.1 carrying - WT1 +/+ isoform	Kindly provided by Professor Dr. Haruo Sugiyama from University Graduate School of medicine, Osaka, Japan.
- WT1 +/- isoform	
- WT1 -/+ isoform	
- WT1 -/- isoform	
Mock control (pcDNA3.1 empty vector)	
WT1 promoter vector -1807 bp/Lec - 907 bp/Lec - 301 bp/Lec - 224 bp/Lec - 203 bp/Lec - 92 bp/Lec	Kindly provided by Professor T Murate from Department of Medical Technology, Nagoya University Graduate School of Health Sciences, Nagoya, Japan
Restriction enzyme EcoRI	New England Biolabs, USA
HindIII	New England Biolabs, USA
Xba I	New England Biolabs, USA
PuvI	TAKARA Bio, Japan

Ligation enzyme T4 DNA ligase	New England Biolabs, USA
Polyclonal antibody PKC isoform antibody sampler kit (Cat # 9960)	Cell Signaling Technology, USA
MAPK Family antibody sampler kit (Cat # 6626)	Cell Signaling Technology, USA
Scr antibody sampler kit (Cat #9935)	Cell Signaling Technology, USA
Stat antibody sampler kit (Cat # 9939)	Cell Signaling Technology, USA
Anti-WT1 (C-19) rabbit polyclonal antibody (Cat # sc-192)	Santa Cruz, USA
Anti-GAPDH (Fl-335) rabbit polyclonal antibody (Cat # sc-25778)	Santa Cruz, USA
Anti-IgG (from Rabbit Serum) (Cat # 15006-10MG)	Sigma-Aldrich, USA
Anti-Sp1 (H225) rabbit polyclonal antibody (Cat # sc-14027x)	Santa Cruz, USA
Rabbit Antiserum To mouse IgG (Whole molecule) (Cat#55436)	MP Biomedical, USA
Monoclonal antibody Anti-actin mouse monoclonal antibody (AC-15) (Cat # A1978)	Sigma-Aldrich, USA
Anti FLAG® M2 mouse monoclonal antibody (Cat#200472-21)	Stratagene, USA

Anti-RNA polymerase II 8WG16 monoclonal antibody (Cat # MMS-126R)	Covance, USA
Anti-GFP mouse monoclonal antibody (Cat # sc-9996)	Santa Cruz, USA
Anti-GATA1 (N6) rat monoclonal antibody (Cat # sc-265x)	Santa Cruz
Secondary antibody Anti rabbit IgG (H+L), HRP conjugate	Promega, USA
Goat anti mouse IgG (H+L), HRP conjugate	Invitrogen, USA
Goat anti rat IgG (H+L), HRP conjugate	Invitrogen, USA
Anti biotin	Invitrogen, USA
Reverse transcriptase Polymerase chain reaction (PCR) reagents WT1 primer Forward : 5'-GGCATCTGAGACCAGTGAGAA-3' Reverse : 5'-GAGAGTCAGACTTGAAAGCAGT-3'	Invitrogen, USA
GAPDH Forward : 5'-CGAAGTCAACGGATTTGGTCGTAT-3' Reverse : 5'-CAGAAGTGGTGGCTGTTCCGA-3'	Invitrogen, USA
WT1 promoter primer #1 Forward primer: 5'-CCTGAACGGACTCTCCAGTG-3' Reverse primer: 5'-CGCTGCCTTGAACTCCTTAC-3'	Operon, USA
WT1 promoter primer #2 Forward primer: 5'-GGCCCCTCTTATTTGAGCTT-3' Reverse primer: 5'-CAAGAGGAAGTCCAGGATCG-3'	Operon, USA

GATA1 promoter primer Forward primer: 5’ GGGAATTCGACTCATTTATATCAGCCG TTTT-3’ Reverse primer: 5’-GGGTCGACCCTGGCTCTTTCCGACTC-3’	IDT, USA
WT1 promoter mutant primer (PAGE purified) Forward primer: 5'-CCTCCCCTTCTTGGCCTTCGCCAATWTG AWATCWTCGTCTCACTGGAGAGTCC-3’ Reverse primer: 5’-GGACTCTCCAGTGAGACGAWGATWTC AWATTGGCGAAGGCCAAGAAGGGGAGG-3’	IDT, USA
SuperScript® One-Step RT-PCR System with Platinum®*Taq* DNA Polymerase	Invitrogen, USA
5X buffer GoTaq	Promega, USA
GoTaq® DNApolymerase (Cat # M3005)	Promega, USA
Deoxyribonucelotide triphosphate (dNTP)	Invitrogen, USA
Betain	Invitrogen, USA
Quantitative PCR WT1 TaqMan probe (Hs01103754_m1)	Applied Biosystem, USA
GAPDH (human)	Applied Biosystem, USA
Actin (human)	Applied Biosystem, USA

Master Mix	Applied Biosystem, USA
High-Capacity cDNA Reverse Transcription kit	Applied Biosystem, USA

Appendix B

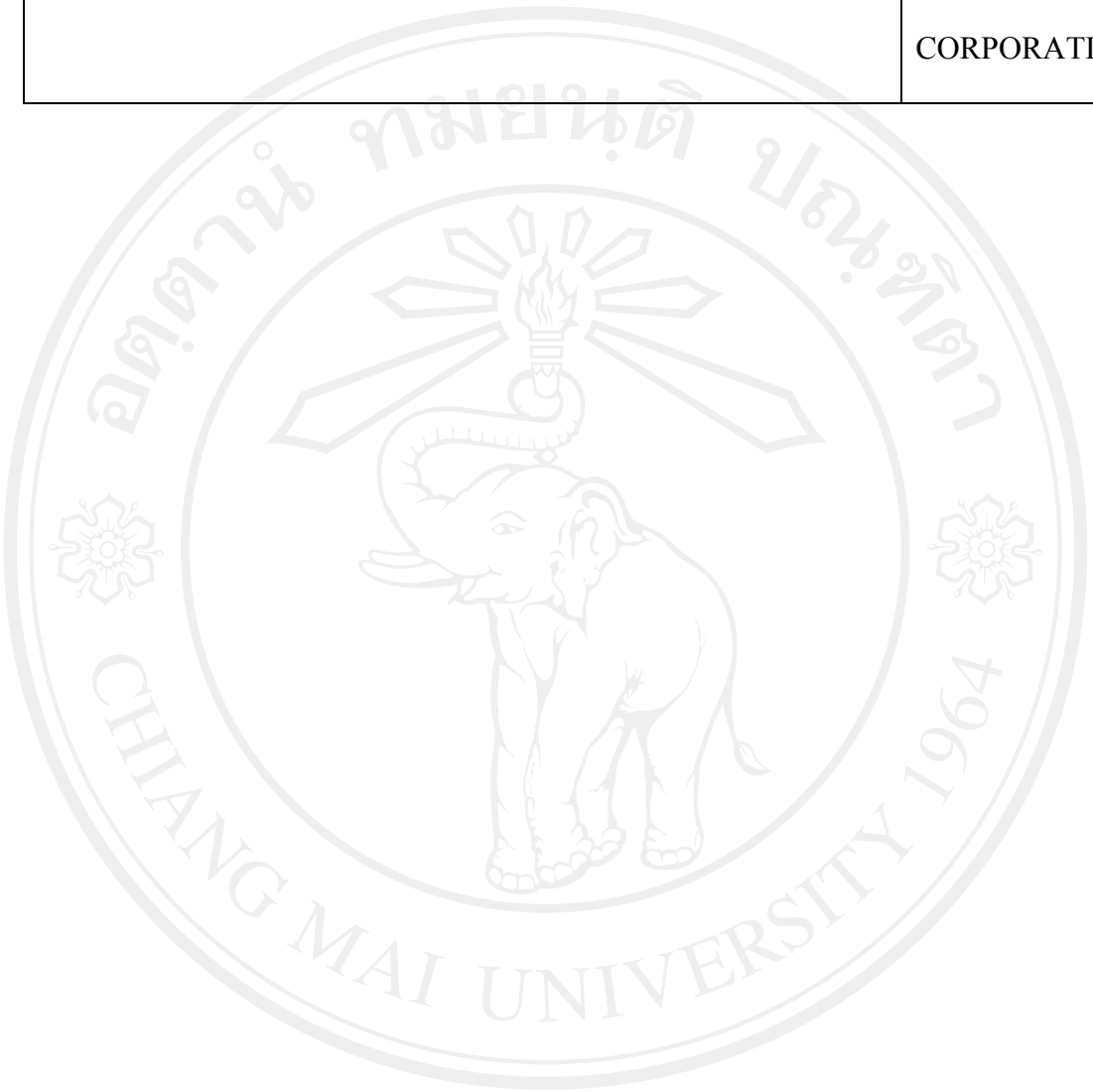
The following equipment used in this study

Name of equipments	Company
Equipment Analytical balance	OHAUS CORPORATION, Thailand
Autoclave	HUXLER, Thailand
Auto-pipette - 1000 µL - 200 µL - 20 µL - 10 µL - 2 µL	BIOHIT, GILSON, LABMATE, and BioRad
Automatic pipette tip	BIOLINE
Carbon dioxide incubator	FORMA SCIENTIFIC
Centrifuge	EPPENDORF 5810
Deionized distilled water machine	PK WATER TEXT
Freezer (-20°C)	SANYO

Gel electrophoresis apparatus	BioRad, USA
Freezer (-80°C)	PTW ULTRA COLD
Gel documentation (Innotech)	Cell Biosciences, USA
GloMax. 96 Microplate Luminometer with Dual Injectors	Promega, USA
ECL-hyper film	PIERCE
Homogenizer	PARGUS (JAPAN)
Inversted microscope	OLYMPUS, USA
10 cm glass plate	PYREX and PETRIO
Plastic plates - 6 well-tissue culture plate - 12 well- tissue culture plate - 24 well- tissue culture plate - 96 well- tissue culture plate - 6 cm tissue culture plate - 10 cm tissue culture plate - 15 cm tissue culture plate	NUNC, USA
iQcycle (qPCR)	BioRad, USA
Larminar flow biological cabinet	CLEAN
Light microscope	OLYMPUS, USA
Nitrocellulose membrane	BioRad, USA
Magnetic stirrer	SYBRON/THERM OLYNE

1.5 mL microcentrifuge tube, 10 and 50 mL centrifuge tube	GREINER BIO-ONE and CORNING INCORPORATION
Microcentrifuge, bench-topped	Eppendorf, USA
Mini protein II slab gel	BioRad, USA
Pasture pipette	PYREX, USA
PCR mechanize	BioRad, USA
Milipore filter paper	PALL CORPORATION, USA
pH meter	THERMO ORION
Power supply	BioRad, USA
Refrigerator	TOSHIBA, Thailand
Serological pipette	PYREX, USA
Spectrophotometer	SHIMADZU, BARA SCIENTIFIC
25 or 75 cm^3 T-flask	NUNC
Trans-blot® electrophoretic transfer protein box	BioRad, USA
Water bath	ISOTEMP
PCR tube dome or flat caps	MOLECULAR BIO PRODUCTS

Vortex mixer	GEMMY INDUSTRIAL CORPORATION



Appendix C

Protocol for reagent preparation

Human leukemic cell lines culture

1. RPMI 1640 medium

RPMI 1640 (powder)	10.4	gm
HEPES	3.57	gm
$NaHCO_3$	2	gm
0.34% 2-Mercaptoethanol	1	mL
Deionized distilled water up to	1000	mL

The solution was filtered with 0.2 μm membrane filter and kept at 4°C.

2. 10% FBS (fetal bovine serum) in RPMI 1640 medium (complete medium)

RPMI 1640 medium	885	mL
FBS	100	mL
Penicillin and streptomycin	10	mL
L-glutamine	5	mL

The solution was kept at 4°C.

3. 0.1% FBS in RPMI 1640 medium (serum starve medium)

RPMI 1640 medium	490	mL
FBS	5	mL
Penicillin and streptomycin	5	mL

The solution was kept at 4^{o}C.

4. Freezing solution (8% DMSO in fetal bovine serum)

Fetal bovine serum	8	mL
DMSO	2	mL

The solution was kept at 4°C.

5. Phosphate buffer saline (PBS) pH 7.4

NaCl	8	gm
KCl	0.2	gm
Na_2HPO_4	1.44	gm
KH_2PO_4	0.24	gm
Deionzied distilled water	800	mL

The pH was adjusted to 7.4 then top up to 1000 mL. Then the solution for cell culture must be filtered with 0.2 μm membrane filter or autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept at room temperature.

Cell viability experiment

6. MTT strock dye solution

MTT dye	0.050	gm
1X PBS	10	mL

The solution was filtered with 0.2 μm membrane filter and then kept at 4°C.

7. 0.2% (W/V) trypan blue

Trypan blue	0.2	gm
PBS	100	mL

The solution was kept at room temperature.

Protein extraction

Whole protein extraction (crude protein)

8. RIPA buffer

50 mM Tris-base	3.03	gm
10% SDS	5	mL
Triton X-100	5	mL
150 mM NaCl	4.38	gm
0.5 mM EDTA	0.093	gm
Deionized distilled water	400	mL

The pH was adjusted to 7.5 by using concentrated HCl. Then deionized distilled water was added up to 500 mL. It was kept at 4°C.

Cytosol and nuclear protein extraction

9. Buffer A

1 M HEPES pH 7.9	2	mL
4.2 M $MgCl_2$	72	µL
KCl	0.1492	gm
0.1 M DTT	1	mL
Deionzied distilled water up to	200	mL

The solution was kept at 4°C. It is stable 1 month.

10. Buffer B (0.1% NP-40 in buffer A)

Buffer A	100	mL
Nonidet P -40 (igepal)	100	µL

The solution should be prepared from individual stock solution.

SDS-PAGE analysis

11. Stock solution A: separating gel buffer 1.5 M Tris-base HCl, pH 8.8

Tris-base	181.7	gm
10% SDS	40	mL

The solution was adjusted to pH 8.8 by concentrated HCl. Then deionized distilled water was added up to 1000 mL. It was kept at room temperature.

12. Stcok solution B: stacking gel buffer 1.0 M Tris-base HCl, pH 6.8

Tris-base	121.2	gm
10% SDS	40	mL

The solution was adjusted to pH 6.8 by concentrated HCl. Then deionized distilled water was added up to 1000 mL. It was kept at room temperature.

13. Stock solution C: stock acryla mide solution (30% T, 2.7%)

Acrylamide	29.2	gm
Bis (Estaman)	0.8	gm
Deionized distilled water up to	70	mL

The solution was adjusted volume to 100 mL and any insoluble powder was removed from the solution by filtration with membrane filter pore size 0.2 μm. It was kept at 4°C in dark container.

14. Stock ammonium persulfate solution

10% (w/v) APS in deionized distilled water

Ammonium persulfate	1	gm
Deionized distilled water up to	10	mL

The solution was aliquoted 1 mL/tube and kept at -20°C.

15. Stock 10% SDS solution

SDS	10	gm
Deionized distilled water up to	100	mL

The solution was kept at room temperature.

16. 10X Running buffer

Tris-base	121.2	gm
Glycine	752	gm
SDS	40	gm
Deionized distilled water up to	4000	mL

The solution was kept at room temperature.

17. 1X Running buffer

10X Running buffer	100	mL
Deionized distilled water up to	1000	mL

The solution was kept at room temperature.

Table 38 The percentage of SDS-PAGE used for preparing gel

Resolving Gel (mL)	**6%**	**8%**	**10%**	**12%**	**15%**
30% Acrylamide	6	8	10	12	15
1.5 M Tris, pH 8.8 + SDS (0.4% at final concentration)	7.5	7.5	7.5	7.5	7.5
Distilled water	16	14	12	10	7
10% APS	0.3	0.3	0.3	0.3	0.3
TEMED	0.024	0.018	0.012	0.012	0.012

4% Stacker (mL)	1 gel	2 gels	4 gels
30% Acrylamide	0.8	1.6	3.2
1.0 M Tris-HCl, pH 6.8 + SDS (0.4% at final concentration)	1.2	3	6
Distilled water	3.7	7.4	14.8
10% APS	0.06	0.1	0.2
TEMED	0.006	0.015	0.02

18. 5X Non-reducing buffer

1.0 M Tris-HCl, pH 6.8	0.625	mL
Glycerol	1.0	mL
1% Bromphenol blue	0.125	mL
Deionized distilled water up to	10	mL

The solution was aliquoted 0.95 mL/tube and kept at -20°C.

19. 5X Reducing buffer

5x non-reducing buffer	0.95	mL
2-mercaptoethanol	0.05	mL

The solution was kept at -20°C.

Western blot analysis

20. 10X Transfer buffer

Tris-base	121.2	gm
Glycine	576	gm
Deionized distilled water up to	4000	mL

The solution was kept at room temperature.

21. 1X Transfer buffer

10X Transfer buffer	100	mL
Methanol	200	mL
Deionized distilled water up to	1000	mL

The solution was kept at room temperature.

22. 10X Washing buffer

NaH_2PO_4	8.16	gm
Na_2HPO_4	52	gm
NaCl	291.2	gm

The pH was adjusted pH to 7.4 by using concentration HCl and then added with 40 mL of Tween 20. Deionized distilled water was added up to 4000 mL.

23. 1X Washing buffer

10X Washing buffer	100	mL
Deionized distilled water up to	1000	mL

The solution was kept at room temperature.

24. Blocking buffer (5% Skim milk)

Skim milk	50	gm
2% NaN_3	10	mL

The solution was adjusted volume to 1000 mL with 1X Washing buffer. It was kept at 4°C.

25. Staining membrane

0.5% ponceau S	0.5	gm
0.1% acetic acid	100	µL
Deionzied distilled water up to	100	mL

The solution was kept at room temperature.

26. Coomasie blue stain

Coomasie blue	0.25	gm
Methanol	20	mL
Acetic acid	10	mL
Deionzied distilled water up to	100	mL

The solution was kept at room temperature.

27. Coomasie blue destaining solution

Methanol	100	mL
Acetic acid	50	mL
Deionzied distilled water up to	500	mL

The solution was kept at room temperature.

Polymerase chain reaction (PCR) experiment

28. 50X TAE

Tris-base	242	gm
EDTA	18.6	gm
Glacial acetic acid	57.1	mL
Deionzied distilled water up to	1000	mL

The solution was kept at room temperature.

29. 1X TAE

50X TAE	10	mL
Deionzied distilled water up to	500	mL

The solution was kept at room temperature.

30. DEPC treated water

Deionized distilled water	4000	mL
Diethylpyrocarbonate (DEPC)	400	µL

The solution was kept at room temperature.

31. 1 mg/mL Ethidium bromide

Ethidium bromide	0.1	gm
DEPC treated water up to	100	mL

The solution was kept at room temperature.

32. 6X Loading dye

Bromphenol blue	0.25	gm
Sucrose	40	gm
DEPC treated water up to	100	mL

Sucrose should be dissolved before adding phenol blue and then stored at 4°C in dark.

Chromatin Immunopicipiation (ChIP) assay

Stock reagents

33. 0.5 M EDTA

EDTA (disodium salt)	18.6	gm
Deionzied distilled water up to	100	mL

The solution was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept in room temperature.

34. 5 M NaCl

NaCl	29.2	gm
Deionzied distilled water up to	100	mL

The solution was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept at room temperature.

35. 5 M LiCl

LiCl	21.2	gm
Deionzied distilled water up to	100	mL

The solution was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept at room temperature.

36. 1 M KCl

KCl	7.45	gm
Deionzied distilled water up to	100	mL

The solution was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept at room temperature.

37. 1 M $NaHCO_3$

$NaHCO_3$	8.4	gm
Deionzied distilled water up to	100	mL

The solution was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept at room temperature.

38. 1 M Tris-HCl, pH 9.0

Tris-base	12.1	gm
Deionzied distilled water up to	70	mL

The pH of solution was adjusted to 9.0 by using concentrated HCl and then adjusted volume to 100 mL with deionized distilled water. It was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept at room temperature.

39. 20 % Sodium dodecyl sulfate (SDS)

SDS	20	gm
Deionzied distilled water up to	100	mL

The solution was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept in room temperature.

40. 1 M Tris-HCl, pH 8.0

Tris-base	12.1	gm
Deionzied distilled water up to	70	mL

The pH of solution was adjusted to 8.0 by concentrated HCl and then adjusted volume to 100 mL with distilled water. It was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept at room temperature.

41. 1 M Tris-HCl, pH 7.6

Tris-base	12.1	gm
Deionzied distilled water up to	70	mL

The pH of solution was adjusted to 7.6 by concentrated HCl and then adjusted volume to 100 mL with distilled water. It was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept at room temperature.

Working Solutions

42. Cell lysis buffer

PIPES	0.17	gm
1 M KCl	8.5	mL
Deionzied distilled water up to	100	mL

The solution was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept at room temperature.

43. Nuclei lysis buffer

1 M Tris-HCl, pH 8.0	5	mL
0.5 M EDTA	2	mL
20% SDS	5	mL
Deionzied distilled water up to	100	mL

The solution was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept at room temperature.

44. IP dilution Buffer

20% SDS	0.05	mL
Triton X 100	1.1	mL
0.5 M EDTA	0.24	mL
1 M Tris-HCl, pH 8.0	1.67	mL
5 M NaCl	3.34	mL
Deionzied distilled water up to	100	mL

The solution was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept at room temperature.

45. 1X Dialysis buffer with sarkosyl (for polyclonal antibodies)

0.5 M EDTA	0.8	mL
1 M Tris-HCl, pH 8.0	10	mL
Sarkosyl	0.4	gm
Deionzied distilled water up to	200	mL

The solution was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept at room temperature.

46. 1X Dialysis buffer without sarkosyl (for monoclonal antibodies)

0.5 M EDTA	2	mL
1 M Tris-HCl, pH 8.0	25	mL
Deionzied distilled water up to	500	mL

The solution was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept at room temperature.

47. IP wash buffer (for polyclonal antibodies)

1 M Tris-HCl, pH 9.0	20	mL
5 M LiCl	20	mL
Nonidet P -40 (igepal)	2	mL
Deoxycholic acid (dry)	2	gm
Deionzied distilled water up to	200	mL

The solution was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept at room temperature.

48. IP wash buffer (for monoclonal antibodies)

1 M Tris-HCl, pH 8.0	20	mL
5 M LiCl	20	mL
Nonidet P -40 (igepal)	2	mL
Deoxycholic acid (dry)	2	gm
Deionzied distilled water up to	200	mL

The solution was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept at room temperature.

49. Elution buffer

1 M $NaHCO_3$	5	mL
20% SDS	5	mL
Deionzied distilled water up to	100	mL

The solution was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept at room temperature.

Gene cloning

50. LB-broth

LB powder	25	gm
Deionzied distilled water up to	1000	mL

The solution was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. It was kept at 4°C.

51. LB-broth with 100 µg/mL Amplicillin

LB-broth	500	mL
Amplicillin	0.5	mL

The solution was kept at 4°C.

52. LB agar

LB agar powder	37	gm
Deionzied distilled water up to	1000	mL

The solution was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. Five-teen milliliter of sterile solution was poured into sterile plate and then kept at 4°C.

53. LB agar with 100 µg/mL Amplicillin

LB agar powder	37	gm
Deionzied distilled water up to	1000	mL

The solution was autoclaved at the condition of 121°C with the pressure of 15 pound/inch^2 for 15 min. After cooling to a temperature of 55°C, 1 mL of Amplicillin was added. The LB agar containing Amplicillin was poured into sterile plate and then kept at 4°C.

54. Transformation buffer

PIPES (final concentration 10 mM)	3.024	gm
$CaCl_2$ (final concentration 15 mM)	2.208	gm
KCl (final concentration 250 mM)	18.64	gm
Deionzied distilled water	900	mL

The solution was adjusted pH up to 6.7 by 10% KOH.

$MnCl_2$	10.9	gm
Deionzied distilled water up to	1000	mL

The solution was filtrated with 0.2 µM membrane filter. It was kept at 4°C.

CURRICULUM VITAE

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Publications

1. **Semsri S**, Ampasavate C, Okonogi S, Srikamchum M, Anuchapreeda S. Effect of Mangosteen peel fraction extracts on *WT1* gene and WT1 protein expression in leukemic cell lines. Songkla Med J. 27(5); 2009: 389-403.

2. **Semsri S**, Krig SR, Kotelawala L, Sweeney C., Anuchapreeda S. Inhibitory mechanism of pure curcumin on *Wilms' tumor 1* (*WT1*) gene expression through the PKCα signaling pathway in leukemic K562 cells. FEBS Letters. 585(14); 2011: 2235-2242.

3. **Semsri S**, Sweeney C, Intasai N, Jomgeow T, Tima S, Limtrakul P, Anuchapreeda S. Pure curcumin inhibits exogenous WT1 (+/+) isoform protein via degradation pathway and protein kinase C in transfected U937 cells. African Journal of Pharmacy and Pharmacology. (In press)

Meeting presentations

1. **Semsri S**, Tima S, Duangrat C, Okonogi S, Srikamchum M, Anuchapreeda S. Inhibitory effect of Mangosteen peel *(Garcinia mangostana*) fraction extracts on *WT1* gene expression in leukemic cell lines. The 32th Annual Meeting of the Association of Medical Techologists of Thailand. Ambassador City Jomtien, Pattaya, Cholburi, Thailand. On May 6th to 9th, 2008. (Poster presentation)

2. **Semsri S**, Srikamchum M, Okonogi S, Duangrat C, Anuchapreeda S. Inhibitory effect of Mangosteen peel *(Garcinia mangostana*) fractional extracts on *WT1* gene expression in leukemic cell lines. The XXXIInd World Congress of The International Society of Hematology. Bangkok, Thailand. On October 19th to 23rd, 2008. (Poster presentation)

3. **Semsri S**, Intasai N, Jomgeow T, Tima S, Anuchapreeda S. Pure curcumin inhibits WT1(+/+) and WT1 (+/-) isoforms in transduced U937 cell line. The 2nd Graduate Students Academic Day. Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand. On November 12th, 2008. (Oral presentation)

4. **Semsri S**, Intasai N, Jomgeow T, Tima S, Anuchapreeda S. Pure curcumin inhibits WT1(+/+) and WT1 (+/-) isoforms in transduced U937 cell line. Annual Meeting of Faculty of Associated Medical Sciences, Chiang Mai University, Lotus Hotel Pang Suan Kaew, Chiang Mai, Thailand. On December 2nd to 4th, 2008. (Poster presentation)

5. **Semsri S**, Jomgeow T, Intasai N, Tima S, Anuchapreeda S. Pure curcumin inhibit WT1 (+/+) WT1 (+/-) isoforms in transduced U937 cell line. The 4th research path innovation for life. On December 19th to 20th, 2008, Chiang Mai University, Chiang Mai, Thailand. (Poster presentation)

6. **Semsri S**, Srikamchum M, Okonogi S, Duangrat C, Anuchapreeda S. Inhibitory effect of Mangosteen peel *(Garcinia mangostana*) fraction extracts on *WT1* gene expression in leukemic cell lines. The 4th research path innovation for life. On December 19th to 20th, 2008, Chiang Mai University, Chiang Mai, Thailand. (Poster presentation)

7. **Semsri S**, Intasai N, Jomgeow T, Tima S, Anuchapreeda S. Effect of Pure curcumin on WT1 (+/+) isoform in transduced U937 cell line. The 2nd Biochemistry and Molecular Biology for regional sustainable development. On May 7th to 8th, 2009, Faculty of Science, Khon KaenUnversity, Khon Kaen, Thailand. (Poster presentation)

8. **Semsri S**, Intasai N, Jomgeow T, Tima S, Anuchapreeda S. Effect of pure curcumin from turmeric on *WT1* gene and WT1 isoform protein in transfected U937 cells. The 33th Annual Meeting of the Association of Medical Techologists of Thailand. The Empress Chiang Mai, Thailand. On April 28th to May 1st 2009. (Poster presentation)

9. **Semsri S**, Sweeney C, Intasai N, Krig S, Kotelawala L, Jomgeow T, Anuchapreeda S. Inhibitory mechanism of curcumin on *Wilms' tumor1* (*WT1*) gene expression in

K562 cell line. The Commission on Higher Education Congress III University Staff Development Consortium (CHE-USDC Congress III). Office of the Higher Education Commission, Pattaya, Cholburi, Thailand. On September, 9th to 11th, 2010. (Poster presentation)

10. **Semsri S**, Anuchapreeda S, Sweeney C, King SR., Kotelawala L. Dietary curcumin from turmeric down-regulates *Wilms'tumor 1* (*WT1*) gene expression through the PKCα signalling pathway in leukemic cells. EuroFoodChem XVI. Gdansk University of Technology, Poland. On July, 6th to 8th, 2011. (Poster presentation)

Award

Excellent oral presentation award:

1. **Semsri S**, Intasai N, Jomgeow T, Tima S, Anuchapreeda S. Pure curcumin inhibits WT1(+/+) and WT1 (+/-) isoforms in transduced U937 cell line. The 2nd Graduate Students Academic Day. Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand. On November 12th, 2008.

Excellent poster presentation award:

1. **Semsri S**, Srikamchum M, Okonogi S, Duangrat C, Anuchapreeda S. Inhibitory effect of Mangosteen peel *(Garcinia mangostana*) fraction extracts on *WT1* gene expression in leukemic cell lines. The 4th research path innovation for life. On December 19th to 20th, 2008, Chiang Mai University, Chiang Mai, Thailand.

2. **Semsri S**, Sweeney C, Intasai N, Krig S, Kotelawala L, Jomgeow T, Anuchapreeda S. Inhibitory mechanism of curcumin on *Wilms' tumor1* (*WT1*) gene expression in K562 cell line. The Commission on Higher Education Congress III University Staff

Development Consortium (CHE-USDC Congress III). Office of the Higher Education Commission, Pattaya, Cholburi, Thailand. On September, 9th to 11th, 2010.

Young Researcher Award

1. **Semsri S**, Anuchapreeda S, Sweeney C, King SR., Kotelawala L. Dietary curcumin from turmeric down-regulates *Wilms'tumor 1* (*WT1*) gene expression through the PKCα signalling pathway in leukemic cells. EuroFoodChem XVI. Gdansk University of Technology, Poland. On July, 6th to 8th, 2011.

Scholarship

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