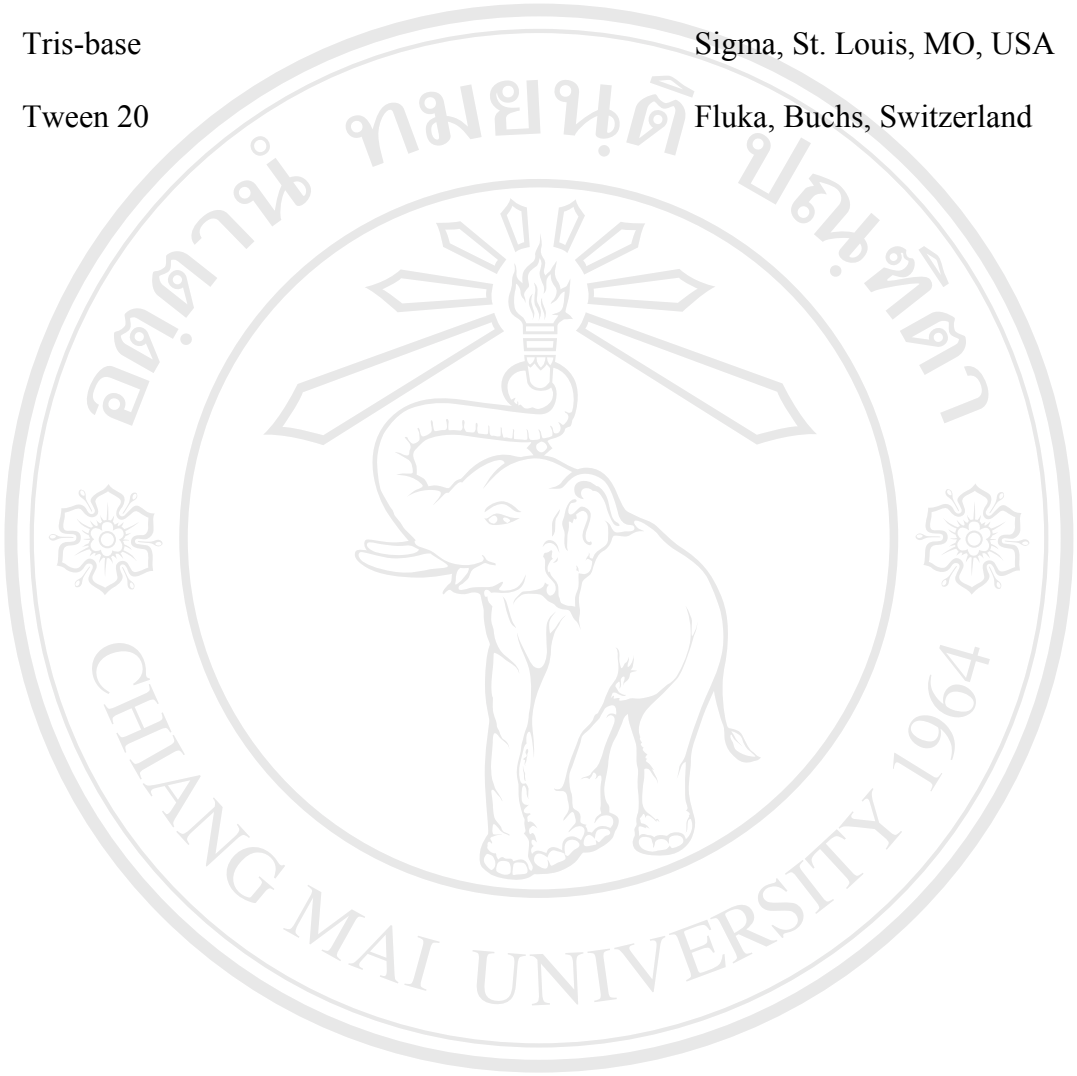
APPENDIX

Appendix A: List of the chemicals and materials used in this study

Chemicals/Materials	**Source**
Acrylamide	Merck, Darmstadt, Germany
ADP reagent	Sigma, St. Louis, MO, USA
Ammonium persulfate	Sigma, St. Louis, MO, USA
Ampicilline	Sigma, St. Louis, MO, USA
Aprotinin	Sigma, St. Louis, MO, USA
Bisacrylamide	Sigma, St. Louis, MO, USA
Bovine serum albumin	Sigma, St. Louis, MO, USA
Chemilumnescent reagent	Pierce, Rockford, IL, USA
Collagen,type IV ; from human placenta	Sigma, St. Louis, MO, USA
Coomassie brilliant blue R-250	Bio-Rad, Hercules, CA, USA
Developer and replenisher	Kodak, NY, USA
Ethyl alcohol	Merck, Darmstadt, Germany
Fetal calf serum	Gibco, Grand Island, NY, USA
Fibrinogen, fraction I, type I: from human plasma	Sigma, St. Louis, MO, USA
Ficoll-Hypaque solution	Sigma, St. Louis, MO, USA
FITC-conjugated sheep $F(ab')_2$ anti-mouse Igs	Silenus, Boronia, Victoria, Australia
Gentamycin	Russel, London, UK
Iodoacetamide	Sigma, St. Louis, MO, USA
Iscove's modified Dulbecco's medium	Gibco, Grand Island, NY, USA
Isostrip Mouse Monoclonal Antibody Isotyping Kit	Roche, Indianapolis, USA

Chemicals/Materials	Source
2-mercaptoethanol	Merck, Darmstadt, Germany
Methanol	Merck, Darmstadt, Germany
Nonidet P-40	Pierce, Rockford, IL, USA
PVDF membrane	PALL, East Hill, NY, USA
Paraformaldehyde	Fluka, Buchs, Switzerland
Potassium chloride	Merck, Darmstadt, Germany
Potassium dihydrogen phosphate	Merck, Darmstadt, Germany
P-Selectin antibody	Santa Cruz Biotechnology, USA
Purified anti-human CD42b	Biolegend, California, USA
Prestained SDS-PAGE standards	Fermentas, MA, USA
Skimmed milk	Difco laboratories, Detroit,MI, USA
Sodium azide	Merck, Darmstadt, Germany
Sodium bicarbonate	Merck, Darmstadt, Germany
Sodium carbonate	Merck, Darmstadt, Germany
Sodium chloride	Merck, Darmstadt, Germany
Sodium dihydrogen phosphate	Merck, Darmstadt, Germany
Sodium dodecyl sulfate	Merck, Darmstadt, Germany
Sodium hydrogen carbonate	Merck, Darmstadt, Germany
Sodium hydrogen phosphate	Merck, Darmstadt, Germany
Sulfo-NHS-LC-biotin	Pierce, Rockford, IL, USA
Sreptavidin-HRP	Zymed, South San Francisco, CA
TEMED	BioRad Laboratories, Griffin

Chemicals/Materials	**Source**
Thrombin	Sigma, St. Louis, MO, USA
Tris-base	Sigma, St. Louis, MO, USA
Tween 20	Fluka, Buchs, Switzerland



Appendix B: List of instruments used in this study

Instruments	**Source**
Autoclave	Huxey, Taiwan
Autopipette	HTL, Poland
Centrifuge	Kendo Laboratory, Germany
CO_2 incubator	Thermo electron corporation, USA
Electrophoresis and Electrotransfer unit	Amersham,,USA
ELISA reader	Tecan, Austria
Flow cytometer-FACSCalibur	Beckton Dickinson, USA
High speed micro refrigerated centrifuge	Tomy, Japan
Inverted microscope	Olympus, Japan
Laminar Flow	Nuaire, USA
Light microscope	Olympus, Japan
Microcentrifuge	Sorvall, Germany
Multichannel autopipette	Socorex, Switzerland
pH meter	Precisa, Switzerland
Refrigerated centrifuge	Sorvall, Germany
Refrigerate (-20°C)	Sanyo, Thailand
Rotator	Technomara, Switzerland
Semi-dry blotting	Amersham Biosciences, Sweden
Spectrophotometer UV-1201	Shimadzu Co., Japan
Water bath	Memmert, Germany

Appendix C: List of hematopoietic cell lines used in this study

Name of cell lines	Type of cell lines
Daudi	Human Burkitt's lymphoma cell line
SupT1	Human acute lymphoblastic leukemia cell line
Jurkat	Human acute lymphoblastic leukemia cell line
Molt4	Human acute lymphoblastic leukemia cell line
HL60	Human promyelocytic leukemia cell line
K562	Erythro-myelocytic cell line
THP1	Human leukemic monocyte lymphoma cell line
U937	Human leukemic monocyte lymphoma cell line

Appendix D: Reagent and buffer preparation

1. Reagents for cell culture

1.1 Incomplete IMDM medium

IMDM powder	1	pack
$NaHCO_3$	3.024	g
Gentamycin (40 mg/ml)	1	ml
Dissolved in ddH_20 and adjust volume to	1000	ml
Filtrated through 0.2 μm Millipore membrane filter		
Added Fungizone (5 mg/ml)	500	μl
Mixed and stored at 4 ^{o}C		

1.2 Complete IMDM medium

Incomplete IMDM medium	90	ml
Fetal calf serum	10	ml
Checked sterility before used		

1.3 RPMI 1640 medium

RPMI powder	1	pack
$NaHCO_3$	2	g
ddH_20	800	ml
Gentamycin (40 mg/ml)	1	ml
Stirred until dissolved and adjust pH with acetic acid		
Dissolved in ddH_20 and adjust volume to 1000 ml		

Filtrated through 0.2 µm Millipore membrane filter

Added Fungizone (5 mg/ml)	500	µl

Mixed and stored at 4 ºC

1.4 Complete RPMI culture medium

RPMI 1640 medium	90	ml
Fetal bovine serum (FBS)	10	ml

Checked sterility before used

1.5 Freezing medium (10%DMSO in 25%FCS-IMDM)

Incomplete IMDM	65	ml
Fetal calf serum	25	ml
DMSO	10	ml

Mixed well and stored at 4°C

1.6 1X HAT medium

Incomplete IMDM	78	ml
Heat-inactivated FCS	10	ml
10X BM condimed HI	10	ml
0.6% 2-ME	30	µl
50X HAT	2	ml

Stored at 4 ºC

1.7 1X HT medium

Incomplete IMDM	119	ml
Heat inactivated FCS	15	ml
BM condimed HI	15	ml
0.6% 2-ME	30	µl
100X HT	1	ml

Mixed well and stored at 4 ºC

1.8 Turk's solution

Glacial acetic acid	3	ml
1% gential violet	1	ml

Adjusted volume to 100 ml with ddH_2O

Filtrated by Whatman filter paper No. 1 and stored at room temperature

1.9 Trypan blue (0.2%)

Trypan blue powder	0.2	g
PBS pH 7.2	100	ml

Filtrated by Whatman filter paper No. 1 and stored at room temperature

2. Reagents for cell lysate preparation

2.1 Tris lysis buffer pH 8.2 (100mM NaCl, 50mM Tris-base, 2 mM EDTA, 0.02% NaN_3)

Tris base	3.03	g
NaCl	2.922	g
EDTA (M.W. 292.25)	0.292	g
NaN_3	0.1	g
Distilled water	200	ml

Adjusted pH to 8.2 by 0.1M NaOH

Adjusted final volume to 500 ml and stored at room temperature

2.2 Lysis buffer

Phenylmethylsulfonyl fluoride (PMSF) (100 mM in acetone)	100	µl
Iodoacetamide (0.5M in distilled water)	100	µl
Aprotinin (1 mg/ml in PBS)	100	µl
Pepstatin A (2mM in DMSO)	10	µl
10% NP-40	1	ml
Tris-lysis buffer pH 8.2	8.7	ml

Mixed well, aliquot into vial and stored at -20 ºC

2.3 1 mM Glycine in PBS

Glycine	0.0375 g
PBS pH 7.2	500 ml

Mixed well and stored at 4ºC

2.4 5 mM Biotin in PBS

Sulfo-NHS-LC-biotin	0.00278 g
PBS pH 7.2	1 ml

Freshly prepared before used

3. Reagents for SDS-PAGE

3.1 4X Separating gel buffer (1.5M Tris HCl pH 8.8)

Tris base	18.15 g
Deionized distilled water	80 ml

Adjusted pH to 8.8 by concentrate HCl

Adjusted final volume to 100 ml

Filtrated 0.2 μm millipore membrane filter

Stored at 4ºC

3.2 4X Stacking gel buffer (0.5M Tris HCl pH 6.8)

Tris base	6.0 g
Deionized distilled water	80 ml

Adjusted pH to 6.8 by concentrate HCl

Adjusted final volume to 100 ml

Filtrated 0.2 µm millipore membrane filter

Stored at 4ºC

3.3 2X non-reducing buffer

0.5 M Tris HCl pH 6.8	2.5	ml
87 % glycerol	2.3	ml
Sodium dodecyl sulfate	0.4	g
Distilled water	5.16	ml
1% Bromphenol blue	40	µl

Mixed well, aliquot and stored at -20ºC

3.4 2X reducing buffer

0.5M Tris HCl pH 6.8	2.5	ml
87% glycerol	2.3	ml
Sodium dodecyl sulfate	0.4	g
Distilled water	4.16	ml
2-ME	1	ml
1% Bromphenol blue	40	µl

Mixed well, aliquot and stored at -20ºC

3.5 1X Running buffer

Tris base	3.028	g
Glycine	14.413	g
Sodium dodecyl sulfate	1.0	g

Distilled water	1000 ml

Mixed well, prepared before used

3.6 30% Monomer (30.8% acrylamide, 2.7% bis-acrylamide)

Acrylamide	60 g
Bis-acrylamide	1.6 g
ddH_2O	200 ml

Mixed well and filtrated through 0.2 µm

Filtered by millipore membrane and kept in dark at 4ºC

3.7 Slab gel

	separating gel			stacking gel
	12.5%	10%	7.5%	4%
Distilled water	3.2 ml	4 ml	4.85 ml	1.5 ml
30% Monomer	4.2 ml	3.3 ml	2.5 ml	332.5 µl
4X Separating gel buffer	2.5 ml	2.5 ml	2.5 ml	-
4X Stacking gel buffer	-	-	-	625 µl
10% SDS (in distilled water)	100 µl	100 µl	100 µl	25 µl
10% APS (in distilled water)	50 µl	50 µl	50 µl	12.5 µl
TEMED	10 µl	10 µl	10 µl	5 µl

3.8 10% APS

Ammonium persulfate	0.1	g
Distilled water	1	ml

Mixed well, aliquot and stored at -20ºC

3.9 10% SDS

Sodium dodecyl sulfate	10	g
Distilled water	100	ml

Mixed well, aliquot and stored at -20ºC

3.10 1X blotting buffer

Tris-base	1.515	g
Glycine	7.205	g
Sodium dodecyl sulfate	0.5	g
Distilled water	350	ml

Mixed well

Methanol	100	ml
Adjusted final volume to	500	ml

Filtrated with 0.2 µm filter, stored at room temperature

3.11 0.025% Coomassie brilliant blue R250

Coomassie brilliant blue R250	0.125	g
Methanol	200	ml
Acetic acid	35	ml

Adjusted volume to 500 ml by dH_2O

Stored at room temperature

4. Reagents for indirect immunofluorescence staining

4.1 1X Phosphate buffer saline (PBS)

NaCl	8	g
KCl	0.2	g
Na_2HPO_4	1.15	g
KH_2PO_4	0.2	g
Distilled water	900	ml

Adjusted pH to 7.2 by 5N NaOH

Adjusted volume to 1000 ml, stored at room temperature

4.2 1%BSA- PBS -0.02%NaN_3

Bovine serum albumin fraction V	10	g
PBS pH 7.2	1000	ml
10% NaN_3 in PBS	2000	µl

Mixed well until BSA completely dissolved, stored at 4ºC

4.3 0.5%BSA- PBS- 0.02%NaN_3

Bovine serum albumin fraction V	5	g
PBS pH 7.2	1000	ml
10% NaN_3 in PBS	2000	µl

Mixed well until BSA completely dissolved, stored at 4ºC

4.4 1%Paraformaldehyde in PBS

Paraformaldehyde	5	g
PBS pH 7.2	500	ml

Warmed at 56ºC until dissolved

Filtrated with 0.2 µm millipore filter, stored at 4ºC

4.5 Hypotonic solution (0.83% NH_4Cl) for RBC lysing

NH_4Cl	0.829	g
$KHCO_3$	0.1	g
EDTA	0.0037	g
Deionized distilled water	90	ml

Adjusted pH to 7.2 with 1N HCl

Adjusted volume to 100 ml

Filtrated 0.4 µm Millipore membrane filter

Stored at 4ºC

5. Reagents for ELISA

5.1 0.05% Tween-PBS

PBS pH 7.2	500	ml
Tween 20	250	µl

Mixed and stored at room temperature

5.2 Blocking buffer (2% BSA-PBS-0.02%NaN_3)

Bovine serum albumin	2	g
PBS pH 7.2	100	ml
10% NaN_3 in PBS	200	µl

Mixed well until BSA completely dissolved, stored at 4ºC

5.3 Stop reaction solution (1N HCl)

Concentrate HCl	8.3	ml
Distilled water	91.7	ml

Slowly dropped HCl to distilled water, stored at room temperature

6. Reagents for IgG purification

6.1 Binding buffer (20 mM sodium phosphate buffer pH 7.0)

1 M Na_2HPO_4	11.6	ml
1 M NaH_2PO_4	8.4	ml
ddH_2O	800	ml

Adjusted the pH to 7.0 with 5 N NaOH

Adjusted the volume to 1000 ml with ddH_2O

Mixed well and filtrated through 0.2 µm millipore membrane filter

Kept at 4 ºC**,** degas for 30 min before used

6.2 Eluting buffer (0.1 M glycine-HCl, pH 2.7)

Glycine	3.753	g
ddH_2O	350	ml

Adjusted the pH to 2.7 with concentrate HCl

Adjusted the volume to 500 ml with ddH_2O

Mixed well and filtrated through 0.2 µm millipore membrane filter

Kept at 4 ºC**,** degas for 30 min before used

6.3 Neutralizing buffer (1 M Tris-HCl, pH 9.0)

Tris-base	12.114	g
ddH_2O	60	ml

Adjusted the pH to 9.0 with concentrate HCl

Adjusted the volume to 100 ml with ddH_2O

Mixed well and filtrated through 0.2 µm millipore membrane filter

Kept at 4 ºC**,** degas for 30 min before used

7. Reagent for purification of mAbs

7.1 1M Na_2HPO_4

Na_2HPO_4	14.2	g
ddH_2O	100	ml

Mixed well and stored at room temperature

7.2 1M NaH_2PO_4

NaH_2PO_4. H_2O	13.8	g
ddH_2O	100	ml

Mixed well and stored at room temperature

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Publication for thesis

1. หนึ่งฤทัย นิ่มนุช และ สาวิตรี เจียมพานิชยกุล. การเปรียบเทียบสารกันเลือดแข็งต่อการเกิดการกระตุ้นของเกล็ดเลือดในหลอดทดลองโดยวิธีโฟลไซโตเมตรี. วารสารเทคนิคการแพทย์เชียงใหม่. ฉบับที่ 2 เดือน พฤษภาคม 2552.

Poster Presentation

1. หนึ่งฤทัย นิ่มนุช, อนุสรณ์ เลขะวิพัฒน์, วัชระ กสิณฤกษ์ และ สาวิตรี เจียมพานิชยกุล. การผลิตและวิเคราะห์คุณลักษณะของโมโนโคลนอล แอนติบอดีต่อโมเลกุลบนผิวของเกล็ดเลือดที่ถูกกระตุ้น. งานประชุมวิชาการประจำปี พ.ศ. 2551 คณะเทคนิคการแพทย์ มหาวิทยาลัยเชียงใหม่ ณ โรงแรมโลตัสปางสวนแก้ว จังหวัดเชียงใหม่ ประเทศไทย วันที่ 2-4 ธันวาคม 2551.