



APPENDIX

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University

All rights reserved

APPENDIX A

LIST OF THE CHEMICALS AND INSTRUMENTS

1. Chemicals

All chemicals used as in this study were analytical grade reagents.

| Chemical name | Source |
|--------------------------------------|--|
| 3,3',5,5'-tetramethylbenzidine (TMB) | Sigma-Aldrich, St. Louis, MO, USA |
| 3-aminopropyltriethoxysilane | Sigma-Aldrich, St. Louis, MO, USA |
| 4',6-diamidino-2-phenylindole (DAPI) | Molecular Probes, Eugene, OR, USA |
| Acrylamide | BDH Laboratory Supplies, UK |
| Agarose (electrophoresis grade) | Sigma-Aldrich, St. Louis, MO, USA |
| Ammonium persulfate | Amersham Pharmacia Biotech, Buckinghamshire, UK |
| Ampicillin | Sigma-Aldrich, St. Louis, MO, USA |
| Bis-acrylamide | BDH Laboratory Supplies, UK |
| Bromophenol blue | Sigma-Aldrich, St. Louis, MO, USA |
| Chemiluminescent detection system | Amersham Pharmacia Biotech, Buckinghamshire, UK |
| CsCl | Roche, IN, USA |
| CSFE | Molecular Probes, Eugene, OR, USA |
| DMEM medium | Gibco, Grand Island, NY, USA |
| EDTA | Sigma-Aldrich, St. Louis, MO, USA |
| Ethanol | Merck, Darmstadt, Germany |

| Chemical name | Source |
|---|--|
| Ethidium bromide | Sigma-Aldrich, St. Louis, MO, USA |
| Fetal bovine serum (FBS) | Gibco, Grand Island, NY, USA |
| Formaldehyde | Sigma-Aldrich, St. Louis, MO, USA |
| FractionPREP™ Cell Fractionation System | BioVision, Mountain View, CA, USA |
| Glacial acetic acid | BDH Laboratory Supplies, UK |
| Glycerol | Sigma-Aldrich, St. Louis, MO, USA |
| Hybond-C membrane | Amersham Pharmacia Biotech, Buckinghamshire, UK |
| IPTG | Amresco, Solon, OH, USA |
| IsoPrep solution | Robbins Scientific Corporation, Sunnyvale, CA, USA |
| MEM medium | Gibco, Grand Island, NY, USA |
| Methanol | Merck, Darmstadt, Germany |
| NaCl | Sigma-Aldrich, St. Louis, MO, USA |
| NaOH | Sigma-Aldrich, St. Louis, MO, USA |
| PEG MW 8000 | Sigma-Aldrich, St. Louis, MO, USA |
| Penicillin/Streptomycin | Gibco, Grand Island, NY, USA |
| Plasmid Mini Kit | QIAGEN, Hilden, Germany |
| Polyvinylidene-fluoride (PVDF) membrane | PALL, East Hills, NY, USA |
| ProofStart DNA polymerase | QIAGEN, Hilden, Germany |

| Chemical name | Source |
|-------------------------------|---|
| QIAprep spin Miniprep kit | QIAGEN, Hilden, Germany |
| QIAquick Gel Extraction kit | QIAGEN, Hilden, Germany |
| QIAquick PCR purification Kit | QIAGEN, Hilden, Germany |
| RPMI 1640 medium | Gibco, Grand Island, NY, USA |
| Skimmed milk | Difco Laboratories, Detroit MI, USA |
| Sodium chloride | Merck, Darmstadt, Germany |
| T ₄ ligase enzyme | Roche Molecular Biochemicals, Manheim, Germany |
| TEMED | BioRad, Hercules, CA, USA |
| Triton X-100 | Sigma-Aldrich, St. Louis, MO, USA |
| Trypan blue 0.4% | Sigma-Aldrich, St. Louis, MO, USA |
| Trypsin-EDTA | Gibco, Grand Island, NY, USA |
| Tween20 | Fluka, Buchs, Switzerland |
| Versene 1× | Gibco, Grand Island, NY, USA |

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
 Copyright© by Chiang Mai University
 All rights reserved

2. Instruments

| Instruments | Source |
|---|---|
| 37 °C incubator | JP Selecta, Barcelona, Spain |
| 37 °C CO ₂ incubator EG 115 IR | Jouan GmbH, Unterhaching, Germany |
| Amicon Ultra centrifugal filter units | Millipore, Cork, Ireland |
| AXIOVISION 4.4 software | Carl Zeiss Canada Ltd., Toronto, ON, Canada |
| Beckman Coulter EPICS ALTRA | Beckman Coulter, Fullerton, CA, USA |
| BECKMAN L-60 ultracentrifuge | Beckman Coulter, Fullerton, CA, USA |
| ELISA plate reader | TECAN, Austria |
| Confocal microscope | Olympus, Japan |
| Electrophoretic power supply 3000Xi | BioRad, Hercules, CA, USA |
| Fluo-Link UV transilluminator | Vilber Lourmat, Marne-la-Vallée Cedex, France |
| FluoVIEW software | Olympus, Japan |
| Inverted fluorescence microscope | Nikon eclipse TE2000-S, Japan |
| Inverted microscope | Olympus, Japan |
| Microcentrifuge | Eppendorf AG, Hamburg, Germany |
| Microplate | NUNC, Roskilde, Denmark |
| MiniVE vertical electrophoresis system | Amersham Pharmacia Biotech, Buckinghamshire, UK |
| MRX-150 Refrigerated microcentrifuge | Tomy Tech USA Inc., CA, USA |
| MyLab orbital shaker OS-20 | BioSan Ltd., Riga, Latvia |

| Instruments | Source |
|-----------------------------------|--|
| RT 6000 D refrigerated centrifuge | Sorvall, Kendro Laboratory Products GmbH, Langenselbold, Germany |
| UV spectrophotometer | Shimadzu Scientific Instruments Inc, Kyoto, Japan |
| UV transilluminator | Fotodyne incorporated, Hartland, WI, USA |
| Vortex-Genie K-550-GE | Scientific Industries Inc, Bohemia, NY, USA |
| Zeiss Apotome microscope | Carl Zeiss Canada Ltd., Toronto, ON, Canada |

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
 Copyright© by Chiang Mai University
 All rights reserved

APPENDIX B

LIST OF MICROORGANISMS

| Microorganism | Source |
|-----------------------------------|---|
| <i>Escherichia coli</i> BJ5183 | Stratagene, CA, USA |
| <i>Escherichia coli</i> DH10B | Stratagene, CA, USA |
| <i>Escherichia coli</i> HB2151 | kindly provided by Dr. A.D. Griffiths, MRC Cambridge, UK |
| <i>Escherichia coli</i> Nova Blue | Novagen, WI, USA |
| <i>Escherichia coli</i> Origami B | Novagen, WI, USA |
| <i>Escherichia coli</i> TG1 | kindly provided by Dr. A.D. Griffiths, MRC Cambridge, UK |
| <i>Escherichia coli</i> XL-1 Blue | Stratagene, CA, USA |
| VCSM13 filamentous phage | Stratagene, CA, USA |

APPENDIX C**LIST OF CELL LINES**

| Name | Type of cell lines |
|-------------|--|
| 293A | Human embryonic kidney cell line |
| Jurkat | Human acute lymphoblastic leukemia cell line |
| HeLa | Human cervical carcinoma cell line |
| HepG2 | Human hepatoma cell line |
| U937 | Human leukemic monocyte lymphoma cell line |

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

APPENDIX D

LIST OF ANTIBODIES AND CONJUGATED ANTIBODIES

| Antibodies name | Source |
|--|--|
| Anti- α V integrin (Cloned L230) | kindly provided by Prof. Dr. Andre Lieber |
| Anti-CAR mAb (Cloned RmcB) | kindly provided by Prof. Dr. Andre Lieber |
| Anti-CD147 mAb (Cloned M6-1B9) | kindly provided by Prof. Dr. Watchara Kasinrerak |
| Anti-gpIII mAb | Exalpha Biologicals, Watertown, MA, USA |
| Anti-gpVIII mAb | Amersham Pharmacia Biotech, Buckinghamshire, UK |
| Anti-mouse Igs-Alexa 488 antibody | Molecular Probes, Eugene, OR, USA |
| Anti-survivin mAb (Cloned MT-SVV3) | kindly provided by Prof. Dr. Watchara Kasinrerak |
| Biotinylated anti- gpIII mAbs | Exalpha Biologicals, Watertown, MA |
| BCCP-2 mAb | kindly provided by Prof. Dr. Watchara Kasinrerak |
| Goat-anti-rat Alexa- Fluor 568 antibody | Molecular Probes, Eugene, OR, USA |
| HRP-conjugated anti- HA antibody | Roche, IN, USA |

| Antibodies name | Source |
|---|---|
| HRP-conjugated anti-gpVIII antibody | Amersham Pharmacia Biotech, Buckinghamshire, UK |
| HRP-conjugated goat anti-mouse Igs antibody | KPL, Gaithersburg, MD, USA |
| Ms-mAb-CD147 (MEM-M6/1) | Abcam, MA, USA |
| PE- conjugated anti CD46 antibody | BD Pharmingen, San Diego, CA, USA |
| Rabbit anti-mouse IgG-HRP | Dako, Dakopatts, High Wycombe, UK |
| Streptavidin-HRP | ZYMED Laboratories, San Francisco, CA, USA |

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
 Copyright© by Chiang Mai University
 All rights reserved

APPENDIX E

REAGENT PREPARATIONS

1. 10× Tris-acetate/EDTA electrophoresis buffer (TAE)

| | |
|---------------------|----------|
| Tris-base | 48.40 gm |
| Glacial acetic acid | 11.42 ml |
| 0.5 M EDTA, pH 8.0 | 20 ml |

Dissolved all ingredients in deionized distilled water and filled up to 1,000 ml.

Sterilized by autoclave and kept at room temperature.

2. 1 or 2 % Agarose gel

| | |
|---------|-----------|
| Agarose | 1 or 2 gm |
| 1× TAE | 100 ml |

Melted by microwave oven until the agarose was completely dissolved.

3. Ethidium bromide working solution (10 mg/ml)

| | |
|------------------|--------|
| Ethidium bromide | 1.0 gm |
| Distilled water | 100 ml |

Dissolved and kept in dark bottle at 4 °C.

4. 6× gel loading buffer

| | |
|-----------------|--------|
| Bromphenol blue | 0.25 % |
|-----------------|--------|

| | |
|----------|------|
| Glycerol | 30 % |
|----------|------|

Mixed thoroughly and stored at -20 °C.

5. Phosphate buffer saline (PBS), pH 7.2

| | |
|------|---------|
| NaCl | 8.00 gm |
|------|---------|

| | |
|-----|---------|
| KCl | 0.20 gm |
|-----|---------|

| | |
|----------------------------------|---------|
| Na ₂ HPO ₄ | 1.15 gm |
|----------------------------------|---------|

| | |
|---------------------------------|---------|
| KH ₂ PO ₄ | 0.20 gm |
|---------------------------------|---------|

Dissolved all ingredients in deionized distilled water and filled up to 900 ml.

Adjusted pH to 7.2 with 1 N HCl or 1 N NaOH.

Added distilled water to adjust the volume to 1,000 ml and kept at room temperature.

6. 50% glucose

| | |
|-----------|------|
| D-glucose | 5 gm |
|-----------|------|

Added distilled water to 10 ml and boiled in boiling water.

Filtered through 0.2 µm Millipore filter and stored at 4 °C.

7. Reagents for using in ELISA

7.1 0.05 M carbonate buffer, pH 9.6

| | |
|--------------------------|----------|
| Na_2CO_3 | 0.159 gm |
| NaHCO_3 | 0.293 gm |
| NaN_3 | 0.02 gm |

Dissolved all ingredients in deionized distilled water and filled up to 90 ml.

Adjusted pH to 9.6.

Adjusted the volume to 100 ml with deionized distilled water and kept at room temperature.

7.2 0.05% Tween20 in PBS (washing buffer for ELISA and Western blot)

| | |
|---------|--------|
| Tween20 | 0.5 ml |
|---------|--------|

Dissolved in 1,000 ml PBS.

Mixed well and stored at room temperature.

7.3 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

8. Reagents for SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

8.1

1.5 M Tris-HCl, pH 8.8

| | |
|-----------|----------|
| Tris-base | 18.15 gm |
|-----------|----------|

Dissolved in 75 ml deionized distilled water.

Adjusted pH to 8.8 with concentrated HCL.

Adjusted the volume to 100 ml with deionized distilled water and stored at 4 °C.

จัดรูปแบบ: สัญลักษณ์แสดงหัวข้อย่อย
และลำดับเลข

8.2

0.5 M Tris-HCl, pH 6.8

| | |
|-----------|--------|
| Tris-base | 6.0 gm |
|-----------|--------|

Dissolved in 75 ml deionized distilled water.

Adjusted pH to 6.8 with concentrated HCL.

Adjusted the volume to 100 ml with deionized distilled water and stored at 4 °C.

จัดรูปแบบ: สัญลักษณ์แสดงหัวข้อย่อย
และลำดับเลข

8.3

Running buffer

| | |
|-----------|---------|
| Tris-base | 1.51 gm |
|-----------|---------|

| | |
|---------|---------|
| Glycine | 7.20 gm |
|---------|---------|

| | |
|------------------------|--------|
| Sodium dodesyl sulfate | 0.5 gm |
|------------------------|--------|

Dissolved in 500 ml deionized distilled water and kept at 4 °C.

จัดรูปแบบ: สัญลักษณ์แสดงหัวข้อย่อย
และลำดับเลข

8.4 Blotting buffer

| | |
|-----------|----------|
| Tris-base | 3.03 gm |
| Glycine | 14.41 gm |
| SDS | 0.5 gm |

Added deionized distilled water to 700 ml and mixed well.

Added 200 ml of methanol

Adjusted the volume to 1,000 ml with deionized distilled water and kept at 4 °C.

จัดรูปแบบ: สัญลักษณ์แสดงหัวข้อย่อย
และลำดับเลข

8.5**Copolymerization of 4% stacking gel (5 ml)**

| | |
|-------------------------|---------|
| Stock acrylamide 30% | 0.83 ml |
| 0.5 M Tris-HCl pH 6.8 | 0.63 ml |
| 10% SDS | 0.05 ml |
| DW | 3.40 ml |
| 10% Ammonium persulfate | 0.05 ml |
| TEMED | 0.01 ml |

จัดรูปแบบ: สัญลักษณ์แสดงหัวข้อย่อย
และลำดับเลข

8.6 Copolymerization of 12% stacking gel (10 ml)

| | |
|-------------------------|---------|
| Stock acrylamide 30% | 4.00 ml |
| Gel buffer pH 8.8 | 2.50 ml |
| 10% SDS | 0.10 ml |
| DW | 3.30 ml |
| 10% Ammonium persulfate | 0.10 ml |
| TEMED | 0.01 ml |

ลิขสิทธิ์ © by Chiang Mai University
All rights reserved

9. Media for bacterial culture

9.1 LB broth

| | |
|---------------|---------|
| Yeast extract | 5.0 gm |
| Tryptone | 10.0 gm |
| NaCl | 10.0 gm |

Dissolved all ingredients in 1,000 ml distilled water.

Sterilized by autoclave, poured on Petri dish (plate) and kept at 4 °C.

9.2 LB agar

| | |
|---------|-------|
| LB agar | 15 gm |
|---------|-------|

Dissolved all ingredients in 1,000 ml distilled water.

Sterilized by autoclave, poured on Petri dish (plate) and stored at 4 °C.

9.3 2×TY broth

| | |
|---------------|-------|
| Tryptone | 16 gm |
| Yeast extract | 10 gm |
| NaCl | 5 gm |

Dissolved in 1,000 ml distilled water.

Sterilized by autoclave and kept at 4 °C.

9.4 TYE

| | |
|---------------|-------|
| Tryptone | 10 gm |
| Yeast extract | 5 gm |
| NaCl | 8 gm |

Dissolved in 1,000 ml distilled water.

Sterilized by autoclave and kept at 4 °C.

9.5 Solid TYE medium

| | |
|---------------|-------|
| Tryptone | 10 gm |
| Yeast extract | 5 gm |
| NaCl | 8 gm |
| Agar | 10 gm |

Dissolved in 1,000 ml distilled water.

Sterilized by autoclave, poured on Petri dish (plate) and kept at 4 °C.

10. Reagents for using in plasmid mini-preparation**10.1 3 M Sodium Acetate pH 7.0**

| | |
|--------------------------|---------|
| NaAcet.3H ₂ O | 40.8 gm |
|--------------------------|---------|

Adjust pH to 7.0 with NaOH/HCl

Dissolve in 100 ml DW and keep at 4 °C

10.2 Potassium Acetate

| | |
|-------------------|---------|
| Potassium Acetate | 29.4 gm |
|-------------------|---------|

| | |
|---------------------|---------|
| Glacial acetic acid | 11.5 ml |
|---------------------|---------|

Dissolve in 100 ml DW and keep at 4 °C.

10.3 10 M NaOH

| | |
|------|--------|
| NaOH | 200 gm |
|------|--------|

Dissolve in 500 ml DW and keep at 4 °C.

10.4 10% SDS

| | |
|-----|------|
| SDS | 5 gm |
|-----|------|

Dissolve in 50 ml DW and store at room temperature.

10.5 7.5 M NH₄ Acetate

| | |
|-------------------------|---------|
| NH ₄ Acetate | 57.8 gm |
|-------------------------|---------|

Dissolve in 100 ml DW and keep at 4 °C.

10.6 1 M glucose buffer

| | |
|-----------|----------|
| D-glucose | 18.02 gm |
|-----------|----------|

Dissolve all ingredients in DW and fill up to 100 ml.

Autoclave and keep at 4 °C.

10.7 0.5 M EDTA pH 8.0

| | | |
|------|-------|----|
| EDTA | 37.22 | gm |
|------|-------|----|

| | | |
|----|-----|----|
| DW | 100 | ml |
|----|-----|----|

Adjust pH to 8.0, add DW to 200 ml and keep at 4 °C.

10.8 10 × glucomix

| | | |
|--------------------|----|----|
| 1 M glucose buffer | 50 | ml |
|--------------------|----|----|

| | | |
|-------------------|----|----|
| 0.5 M EDTA pH 8.0 | 20 | ml |
|-------------------|----|----|

| | | |
|-----------------|----|----|
| 1 M Tris pH 8.0 | 25 | ml |
|-----------------|----|----|

| | | |
|----|---|----|
| DW | 5 | ml |
|----|---|----|

Autoclave and keep at 4 °C.

10.9 1 × glucomix-lysozyme solution

| | | |
|---------------|-----|----|
| 10 × glucomix | 300 | μl |
|---------------|-----|----|

| | | |
|--------------------------------|-----|----|
| Lysozyme stock (50mg/ml in DW) | 300 | μl |
|--------------------------------|-----|----|

| | | |
|----|-----|----|
| DW | 2.4 | ml |
|----|-----|----|

Keep on ice or store at 4 °C for 7 days.

10.10 0.1 M CaCl₂

| | | |
|-------------------|------|----|
| CaCl ₂ | 1.11 | gm |
|-------------------|------|----|

Dissolved in 100 ml distilled water.

Sterilized by autoclave and kept at 4 °C.

10.11 85% glycerol

| | | |
|----------|------|----|
| Glycerol | 42.5 | ml |
|----------|------|----|

Added distilled water to 100 ml.

Mixed well, sterilized by autoclave and stored at room temperature.

11. Reagents for indirect immunofluorescence staining**11.1 Phosphate buffer saline (PBS)**

| | | |
|----------------------------------|------|----|
| NaCl | 8 | gm |
| KCl | 0.2 | gm |
| Na ₂ HPO ₄ | 1.15 | gm |
| KH ₂ HPO ₄ | 0.2 | gm |
| DW | 900 | ml |

Adjusted pH to 7.2 by 5N NaOH

Adjusted volume to 1000 ml, stored at room temperature.

11.2 1% BSA-0.02% NaN₃ in PBS

| | | |
|---------------------------------|------|----|
| Bovine serum albumin fraction V | 10 | gm |
| PBS pH 7.2 | 1000 | ml |
| 10% NaN ₃ in PBS | 200 | μl |

Mixed well until BSA completely dissolved, stored at 4°C.

11.3 1% Paraformaldehyde in PBS

| | | |
|--|-----|----|
| Paraformaldehyde | 5 | gm |
| PBS pH 7.2 | 500 | ml |
| Heat at 56°C until dissolved | | |
| Filtrated with 0.2 µm Millipore filter, stored at 4°C. | | |

12. Reagents for cell culture**12.1 DMEM medium**

| | | |
|---|-----|------|
| DMEM powder | 1 | pack |
| NaHCO ₃ | 3.7 | gm |
| ddH ₂ O | 800 | ml |
| Penicillin (10,000 units/ml)/Streptomycin (10,000 µg/ml) | 1 | ml |

Stirred until dissolved and adjust pH with acetic acid.

Dissolved in ddH₂O and adjust volume to 1,000 ml.

Filtrated through 0.2 µm Millipore membrane filter.

Mixed and stored at 4 °C.

12.2 Complete DMEM culture medium

| | | |
|--------------------------------|----|----|
| DMEM medium | 90 | ml |
| Fetal bovine serum (FBS) | 10 | ml |
| Checked sterility before used. | | |

12.3 Incomplete IMDM medium

| | | |
|-----------------------|-------|------|
| IMDM powder | 1 | pack |
| NaHCO ₃ | 3.024 | gm |
| Gentamycin (40 µg/ml) | 1 | ml |

Dissolved in ddH₂O and adjust volume to 1,000 ml

Filtrated through 0.2 µm Millipore membrane filter.

Mixed and stored at 4 °C.

12.4 Complete IMDM medium

| | | |
|------------------------|----|----|
| Incomplete IMDM medium | 90 | ml |
| Fetal calf serum | 10 | ml |

Checked sterility before used

12.5 RPMI 1640 medium

| | |
|---|--------|
| RPMI powder | 1 pack |
| NaHCO ₃ | 2 gm |
| ddH ₂ O | 800 ml |
| Penicillin (10,000 units/ml)/Streptomycin (10,000 µg/ml) | 1 ml |

Stirred until dissolved and adjust pH with acetic acid.

Dissolved in ddH₂O and adjust volume to 1,000 ml.

Filtrated through 0.2 µm Millipore membrane filter.

Mixed and stored at 4 °C.

12.6 Complete RPMI culture medium

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

Checked sterility before used.

12.7 Freezing medium (10%DMSO in 90%FCS)

| | |
|------------------|------|
| Fetal calf serum | 9 ml |
| DMSO | 1 ml |

Freshly preparation before use.

12.8 Turk's solution

| | | |
|---|---|----|
| Glacial acetic acid | 3 | ml |
| 1% gentian violet | 1 | ml |
| Adjusted volume to 100 ml with ddH ₂ O | | |
| Filtrated by Whatman filter paper No. 1 and stored at room temperature. | | |

12.9 Trypan blue (0.2%)

| | | |
|---|-----|----|
| Trypan blue powder | 0.2 | gm |
| PBS pH 7.2 | 100 | ml |
| Filtrated by Whatman filter paper No. 1 and stored at room temperature. | | |

13. Reagents for adenovirus preparation**13.1 Cesium chloride gradient****Density 1.50 gm/cm³**

| | | |
|------|-------|----|
| CsCl | 45.41 | gm |
| DW | 54.49 | ml |

Density 1.35 gm/cm³

| | | |
|------|-------|----|
| CsCl | 35.18 | gm |
| DW | 64.82 | ml |

Density 1.25 gm/cm³

| | | |
|------|-------|----|
| CsCl | 26.99 | gm |
| DW | 73.01 | ml |

13.2 Dialysis buffer

10 mM Tris pH 7.5

10 mM MgCl₂

10% glycerol

Stored at 4 °C.

13.3 Pronase stock

5 mg/ml Pronase

10 mM Tris pH 7.5

Preincubated at 56 °C for 15 min and followed by 37 °C for 1 h.

Aliquotted and stored at -20 °C.

13.4 Pronase buffer

10 mM Tris HCl pH 7.4

10 mM EDTA pH 8.0

1% SDS

Stored at room temperature.

13.5 TE pH 8.0

10 mM Tris HCl pH 8.0

1 mM EDTA pH 8.0

Stored at room temperature.

14. Denaturing RNA electrophoresis**14.1 0.5 M EDTA, pH 8.0**

| | |
|------|----------|
| EDTA | 186.1 gm |
|------|----------|

| | |
|--------------------------|----------|
| Deionize distilled water | 1,000 ml |
|--------------------------|----------|

Sterile by autoclave.

14.2 DEPC treated water

| | |
|-----------------------------|-------------|
| Diethylpyrocarbonate (DEPC) | 400 μ l |
|-----------------------------|-------------|

| | |
|--------------------------|----------|
| Deionize distilled water | 4,000 ml |
|--------------------------|----------|

Shake it vigorously and store at room temperature, overnight.

Sterile by autoclave.

14.3 5 \times formaldehyde gel running buffer

| | |
|------|---------|
| MOPS | 20.6 gm |
|------|---------|

| | |
|-----------------------------|--------|
| 50 mM Sodium acetate pH 7.0 | 800 ml |
|-----------------------------|--------|

Adjust pH by 2N NaOH

| | |
|-------------------|-------|
| 0.5 M EDTA pH 8.0 | 10 ml |
|-------------------|-------|

Adjust volume to 1,000 ml with DEPC-treated water.

Incubate at room temperature for 24 h and store at 4 °C.

Sterile by autoclave.

14.4 50 mM Sodium acetate pH 7.0

| | |
|--------------------|---------|
| Sodium acetate | 3.28 ml |
| DEPC-treated water | 800 ml |

14.5 1% formaldehyde gel

| | |
|---------------------------------|---------|
| Agarose | 1 gm |
| DEPC-treated water | 62.5 ml |
| 5 × formaldehyde running buffer | 19.6 ml |
| Formaldehyde | 17.9 ml |

15. Reagent for phage precipitation**15.1 PEG/NaCl**

| |
|-------------|
| 4% PEG-8000 |
| 3% NaCl |

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
 Copyright© by Chiang Mai University
 All rights reserved

APPENDIX F**ADDITIONAL FILES****Additional file 1**

Colocalization of CD147 and intrabody. Colocalization of CD147 (red) and intrabody (white) in the transfected 293A cell was demonstrated in pink. Image acquisition and analysis is as described in Materials and Methods.

Additional file 2

Colocalization of CD147 and intrabody. Colocalization of CD147 (green) and intrabody (red) in the transduced HeLa cell was demonstrated in yellowish-orange. Image acquisition and analysis is as described in section 3.8.2.

APPENDIX G**PRESENTATION AND PUBLICATIONS****List of presentation**

1. Intracellular expression of a single-chain variable fragment against CD147 inhibiting surface expression of CD147 in 293A cell. The 18th Annual Meeting of the Thai Society for Biotechnology on “Biotechnology: Benefits & Bioethics” at The Montein Riverside Hotel, Bangkok, Thailand, November 2-3, 2006 (Poster presentation).
2. Controlling of CD147 surface molecule expression by intrabody strategy. The Academic Meeting in The 12th Year Anniversary of the Faculty of Allied Health Sciences at Thammasat University, Pratumthani, Thailand, February 20, 2008 (Oral presentation).
3. Efficient inhibition of OKT3-induced T cell proliferation and suppression of CD147 cell surface expression in HeLa cells by scFv-M6-1B9. The 33rd Annual Meeting of the Association of Medical Technologists of Thailand, at The Empress Hotel, Chiang Mai, Thailand, April 29- May 1, 2009 (Poster presentation).

List of publications

1. Tragoolpua K, Intasai N, Kasinrerak W, Mai S, Yuan Y, Tayapiwatana C. Generation of functional scFv intrabody to abate the expression of CD147 surface molecule of 293A cells. *BMC Biotechnol.* 2008 Jan 29;8:5.

Impact factor 2.38

2. Intasai N, Tragoolpua K, Pingmuang P, Khunkaewla P, Moonsom S, Kasinrerak W, Lieber A, Tayapiwatana C. Potent inhibition of OKT3-induced T cell proliferation and suppression of CD147 cell surface expression in HeLa cells by scFv-M6-1B9. *Immunobiology.* 2009 Mar 3.

Impact factor 3.461

3. Tuve S, Liu Y, Tragoolpua K, Jacobs JD, Yumul RC, Li ZY, Strauss R, Hellström KE, Disis ML, Roffler S, Lieber A. *In situ* adenovirus vaccination engages T effector cells against cancer. *Vaccine.* 2009 Jun 24;27(31):4225-39.

Impact factor 3.298

CURRICULUM VITAE

| | |
|---------------------------|--|
| Name | Mr. Khajornsak Tragoolpua |
| Date of birth | June 19, 1970 |
| Place of birth | Phichit province, Thailand |
| Address | 199/150 Moo 3, Tambol Mae-Hia, Amphur Muang, Chiang Mai 50100 Tel 084-0411600 |
| Education | |
| 1985-1987 | High school, Taphanhin School, Phichit, Thailand. |
| 1988-1991 | Bachelor of Science (Medical Technology), Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand |
| 1993-1996 | Master of Science (Biology), Faculty of Science, Chiang Mai University, Chiang Mai |
| Employment history | |
| Jan 2003 to present | Assistant Professor at Department of Clinical Microbiology, Faculty of Associated Medical Sciences Chiang Mai University, Chiang Mai, Thailand |
| Aug 1996 to Dec 2002 | Lecturer at Department of Clinical Microbiology Faculty of Associated Medical Sciences Chiang Mai University, Chiang Mai, Thailand |
| Jun 1993 to Feb 1994 | Head of Central Laboratory Section, Central Chiang Mai Memorial Hospital, Chiang Mai, Thailand |

Nov 1991 to May 1993 Head of Central Laboratory Section, Ruampath
Chiang Mai Hospital, Chiang Mai, Thailand

Apr 1991 to Oct 1991 Medical Technologist, Radioimmunoassay company,
Bangkok, Thailand

List of publications

1. Tragoolpua K, Intasai N, Kasinrerk W, Mai S, Yuan Y, Tayapiwatana C. Generation of functional scFv intrabody to abate the expression of CD147 surface molecule of 293A cells. *BMC Biotechnol.* 2008 Jan 29;8:5.
2. Intasai N, Tragoolpua K, Pingmuang P, Khunkaewla P, Moonsom S, Kasinrerk W, Lieber A, Tayapiwatana C. Potent inhibition of OKT3-induced T cell proliferation and suppression of CD147 cell surface expression in HeLa cells by scFv-M6-1B9. *Immunobiology.* 2009 Mar 3.
3. Tuve S, Liu Y, Tragoolpua K, Jacobs JD, Yumul RC, Li ZY, Strauss R, Hellström KE, Disis ML, Roffler S, Lieber A. *In situ* adenovirus vaccination engages T effector cells against cancer. *Vaccine.* 2009 Jun 24;27(31):4225-39.