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APPENDICES

APPENDIX I

CHEMICAL AGENTS. MATERIAL. INSTRUMENTS.

1. Chemical agents.

Chemical

Typtose phosphate broth (Sigma, USA)
Lcibovitz-15 (Gibco BRL[®], USA)
Penicillin G (Sigma, USA)
Streptomycin sulfate (Sigma, USA)
Medium 199 (Gibco BRL[®], USA)
Hepes (Sigma, USA)
Sodium hydrogen carbonate (Merck, USA)
L-glutamine (Gibco BRL[®], USA)
Fetal bovine serum (Gibco BRL[®], USA)
Sodium chloride (Merck, USA)
Potassium chloride (Merck, USA)
Absolute ethanol (Merck, USA)
Acrylamide (Pharmacia Biotech, Sweden)
Deoxynucleotide triphosphate (Promega corporation, USA)
Tris-(Hydroxyaminomethyl) aminomethane (Merck, USA)
Ethylene diaminetetracetic acid (Biorad, USA)
Loading dye (Promega corporation, USA)
Agarose (Promega corporation, USA)
4-chloro-1-naphthol (Sigma, USA)
Ethidium bromide (Merck, USA)
Qiagen purification kit (QIAGEN, USA)

Ammonium persulfate (Sigma, USA)
N,N'-methylene-bis-acrylamide (Sigma, USA)
Sodium dodecyl sulfate (Sigma, USA)
N,N,N',N'-tetramethyl-ethylenediamine(TEMED) (Sigma, USA)
QIAamp Viral RNA Minikit (QIAGEN, USA)
100 bp +1100 bp DNA marker (Sib Enzyme, Switzerland)
Broad range protein marker (BioLab[®] Inc, New England)
HRP conjugated goat anti- rabbit IgG (KPL[®], UK)
HRP conjugated mouse anti-human IgG (DAKO, Denmark)
FITC conjugated swine anti-rabbit IgG (DAKO, Denmark)
FITC conjugated mouse anti-human IgG (DAKO, Denmark)
Tween-20 (Merck, USA)
Orthophenylenediamine (Sigma, USA)
Hydrogen peroxide (Merck, USA)
BigDye[™] Terminator Cycle Sequencing reaction kit (Perkin Elmer, USA)
The other chemicals and solvents used were purchased from Gibco BRL[®],

Merck, Sigma, QIAGEN, Promega corporation. Most of them were analytical grade.

2. Enzymes

Taq DNA polymerase (Promega corporation, USA)
RNasin[®]Ribonuclease Inhibitor (Promega corporation, USA)
M-MLV-RT (Promega corporation, USA)

3. Oligonucleotide primers

The primers for PCR protocol and sequencing were purchased from BioBasic Inc., Canada. All primer sequences are listed in Table 2.

4. Instruments

Automatic Micropipette and tip (Gilson Medical Electronic, France)

Polaroid film 677 (Polaroid corporation, USA)

Polaroid DS-34 camera (Polaroid corporation, USA)

Waterbath (Luada, USA)

Centrifuge (Sigma, USA)

Incubator (Forma Scientific, USA)

PCR Thermal cycler: Perkin-Elmer 2400 (PE Applied Biosystem, USA)

ABI 310 Genetic Analyzer (PE Applied Biosystem, USA)

UV transilluminator (Ultra-lum, USA)

-20 °C Freezer (Sanyo, Japan)

-70 °C Freezer (Sanyo, Japan)

pH meter (ORION, USA)

Spectrophotometer (PE Applied Biosystem, USA)

ELISA microplate reader (Organon Teknika, Belgium)

ELISA microplate washer (Organon Teknika, Belgium)

Sonicator (Misonix, USA)

5. Miscellaneous materials

Disposable syringe (Turumo[®], Japan)

Needle gauge, (Turumo[®], Japan)

No.1 filter paper, (Whatman[®], UK)

Microtiter plate, NUNCLON[®], Denmark)

The other plastic wares used were purchase from Costa, NUNCLON and Invitrogen.

APPENDIX II

REAGENT PREPARATION PROTOCOL

I. Media preparation for C6/36 cell cultivation.

1. Growth medium

An enough amount of ingredients for a 100 ml medium is composed of

1. heat inactivated FBS 10 ml (GibcoBRL, USA)
2. TPB 10 ml (Sigma, USA)
3. antibiotics solution 1.2 ml (Sigma, USA)
4. L-15 to the total volume 100 ml (GibcoBRL, USA)

The medium is prepared by mix all component well and store at 4 °C until use.

2. Maintenance medium

An enough amount of ingredients for a 100 ml medium is composed of

1. heat inactivated FBS 1 ml (GibcoBRL, USA)
2. TPB 10 ml (Sigma, USA)
3. antibiotics solution 1.2 ml (Sigma, USA)
4. L-15 to the total volume 100 ml (GibcoBRL, USA)

The medium is prepared by mix all component well and store at 4 °C until use.

3. Antibiotic solutions

The solution consisted of Penicillin G (Sigma, USA) 60.79 U/ml and Streptomycin sulfate (Sigma, USA) 60 µg/ml, both are concentration after adding into the medium. The solution was steriled by though 0.22 µm membrane and store at -20 °C until use.

4. Fetal bovine serum (FBS)

FBS is inactivated by incubated at 56 °C for 30 min and keep at 4 °C until use.

5. Tryptose phosphate broth (TPB)

Dissolved TPB 29.5 gm in distilled water 1L. steriled by autoclave at 121 °C for 15 min and store at room temperature until use.

6. Leibovitz15 (L-15)

Dissolved one pack of L-15 in distilled water 1 L, steriled by though 0.22 µm membrane and store at 4 °C until use.

II. Reagents preparation for SDS-PAGE and Western blot analysis.

1. 30% acrylamide mix

An enough amount of ingredients for a 100 ml 30% acrylamide mix is composed of

- | | |
|------------------------------------|-----------|
| 1. acrylamide | 29% (w/v) |
| 2. N,N' – methylene-bis-acrylamide | 1% (w/v) |

Dissolved both chemical in deionized warm water. Store at 4 °C until use.

2. 1x SDS gel-loading buffer

The buffer is composed of

1. 50mM Tris-Cl (pH 6.8)
2. 100 mM dithiothreitol
3. 2% (w/v) SDS
4. 0.1% bromophenol blue
5. 10% (v/v) glycerol

Dissolved all chemical in deionized water. Store at room temperature until use.

3. Destained solution

The solution is composed of

- | | |
|------------------------|--------|
| 1. Methanol | 500 ml |
| 2. Glacial acetic acid | 100 ml |
| 3. Distilled water | 400 ml |

The solution is prepared by mix all component well and store at room temperature until use.

4. Stained solution

The solution is composed of

- | | |
|-----------------------------------|---------|
| 1. Coomassie Brilliant Blue R-250 | 0.25 gm |
| 2. Destained solution | 100 ml |

Mixed both chemical in together and store at room temperature until use.

5. 10% SDS

The solution is composed of

- | | |
|--------|---------|
| 1. SDS | 10.0 gm |
|--------|---------|

Dissolved the chemical in deionized water and store at room temperature.

6. 10% APS

The solution is composed of

- | | |
|--------|---------|
| 1. APS | 10.0 gm |
|--------|---------|

Dissolved the chemical in deionized water and store at 4 °C until use.

7. Tris-glycine (pH 8.3)

The buffer is composed of

- | |
|--------------------|
| 1. 25 mM Tris-base |
|--------------------|

2. 250 mM glycine
3. 0.1% SDS

Dissolved all chemical in deionized water and adjust pH 8.3 with HCl. Store at room temperature until use.

8. 1 M Tris buffer

The buffer is composed of

- | | |
|--------------|----------|
| 1. Tris base | 121.1 gm |
|--------------|----------|

Dissolved the chemical in deionized water and adjust desired pH with HCl. Adjusted total volume to 1L with deionized water. Store at room temperature until use.

9. Transfer buffer (pH8.3)

An enough amount of ingredients for a 1 L buffer is composed of

- | | |
|--------------|---------|
| 1. Tris-base | 3.0 gm |
| 2. glycine | 14.4 gm |
| 3. methanol | 200 ml |

Dissolved all chemical in deionized water and adjust pH 8.3 with HCl. Store at room temperature until use.

10. 4 M NaCl

An enough amount of ingredients for a 1 L solution is composed of

- | | |
|---------|-----------|
| 1. NaCl | 233.76 gm |
|---------|-----------|

Dissolved the chemical in deionized water and store at room temperature.

11. Washing buffer

An enough amount of ingredients for a 1 L buffer is composed of

1. 10 mM Tris pH 7.2

2. 0.15 M NaCl
3. 0.05% (V/V) Tween-20

Dissolved all chemical in deionized water and store at room temperature.

12. Blocking buffer

An enough amount of ingredients for a 1 L buffer is composed of

1. 10 mM Tris pH 7.2
2. 0.15 M NaCl
3. 5% (W/V) skimmed milk

Dissolved all chemical in deionized water and store at room temperature.

13. Substrate for peroxidase

An enough amount of ingredients for a 30 ml solution is composed of

- | | |
|--------------------------------------|-------|
| 1. 4-chloro-1 naphthol | 15 mg |
| 2. methanol | 5 ml |
| 3. 1 M Tris-Cl pH 7.2 | 25 ml |
| 4. 30% H ₂ O ₂ | 5 µl |

Dissolved all chemical in deionized water to make a total volume to 30 ml and store at room temperature until use.

14. Amido black solution

An enough amount of ingredients for a 30 ml solution is composed of

- | | |
|------------------------|--------|
| 1. Amido black | 1 gm |
| 2. Isopropanol | 250 ml |
| 3. glacial acetic acid | 100 ml |

Dissolved all chemical in deionized water to make a total volume to 1 L and store at room temperature until use.

BIOGRAPHY

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