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ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX I

CHEMICAL AGENT AND INSTRUMENTS

A. Chemicals

Agarose gel (Seakem ME, FMC, Bioproduct, USA)

Aminocaproic acid (Sigma, USA)

Barbitone (BDH, England)

Carboxypeptidase B (Sigma, USA)

Coomassie brilliant blue (Sigma, USA)

Calcium chloride (CaCl_2) (E. Merck, W. Germany)

Diaminobenzidine (DAB) (Sigma, USA)

Disodium hydrogen phosphate (Na_2HPO_4) (E. Merck, W. Germany)

Ethylene diamine tetra acetic acid (EDTA) (E. Merck, W. Germany)

Glacial acetic acid (CH_3COOH) (E. Merck, W. Germany)

Glutaraldehyde (Sigma, USA)

Gelatin (BDH, England)

Glycine (Sigma, USA)

Hydrazine sulfate (Sigma, USA)

Hydrochloric acid (Sigma, USA)

Magnesium chloride (MgCl_2) (E. Merck, W. Germany)

Methanol (CH_3OH) (E. Merck, W. Germany)

Nitrocellulose membrane (Bio-Rad, USA)

Neuraminidase from *Clostridium perfringens* (Type VIII, Sigma, USA)

Non-fat dry milk

Sodium barbital (E. Merck, W. Germany)

Sodium chloride (NaCl) (E. Merck, W. Germany)

Sodium dihydrogen phosphate (E. Merck, W. Germany)

**B. Antiserum and serum**

Guinea pig serum

Hemolysin (Amboceptor) (Behringwerke, W. Germany)

Rabbit immunoglobulin to human C4 (DAKO, Denmark)

Peroxidase-conjugated swine immunoglobulins to rabbit immunoglobulins (DAKO, Denmark)

C. Glassware

Beaker (Pyrex, Corning, NY., USA)

Cylinder (Witeg, West Germany)

Erlenmayer flask (Pyrex, Corning, NY., USA)

Glass tube (Pyrex, Corning, NY., USA)

D. Instrument

Automatic pipette (Finpipette, Helsinki, Finland)

Bio-freezer (Forma, Scientific, USA)

Centrifuge (IEC, CENTRA-7R, Needham Hb., MA, USA)

Electrophoresis unit (LKB, 2117, Sweden)

Incubator (Mammert, W. Germany)

Multiphor II (2219 LKB Sweden) Thermastate Circulator

pH-meter PHM 83 (Radiometer, Copenhagen, Denmark)

Power supply (LKB 2197, Sweden)

Spectrophotometer, Coleman Junior II, model 6135 (USA)

Trans-blot Electrophoretic Transfer cell (Sartatoblot, W. Germany)

Ultrascan XL Laser Densitometer (LKB, 2202, Sweden)

Water bath, Julabo TWA (Seelbach, W. Germany)

APPENDIX II

1. Reagents for C4 Allotyping

1.1 EDTA solution 0.2 M, pH 7.4

Disodium ethylene diamine tetracetate (Na_2 EDTA) 7.44 gm

Distilled water to 100 ml

The pH was adjusted to 7.4 and store at 4°C

1.2 Electrode Buffer (Tris-Glycine-Barbital buffer), pH 8.8

5.5 - diethylbarbituric acid 2.00 gm

5.5 - diethylbarbituric acid - sodium salt 13.30 gm

Tris (hydroxymethyl) - aminomethane 45.20 gm

Glycine 56.20 gm

Distilled water to 2000 ml

Store at 4°C

1.3 Gel Buffer (Tris-Glycine-Barbital buffer), pH 8.8

5.5 - diethylbarbituric acid 0.50 gm

5.5 - diethylbarbituric acid - sodium salt 3.30 gm

Tris (hydroxymethyl) - aminomethane 11.30 gm

Glycine 14.05 gm

Distilled water to 1000 gm

Store at 4°C

1.4 Phosphate buffer 0.1 M - EDTA 4 mM, pH 6.8

| | |
|---|----------|
| Sodium dihydrogen phosphate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) | 6.56 gm |
| Disodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$) | 13.75 gm |
| Disodium EDTA | 1.88 gm |
| Distilled water | 1000 ml |
| Store at 4°C | |

2. Reagent for Staining

2.1 Stain solution

| | |
|--------------------------|---------|
| Coomassie Brilliant Blue | 2.5 gm |
| Methanol | 450 ml |
| Acetic acid | 100 ml |
| Distilled water to | 1000 ml |

2.2 Destain solution

| | |
|--------------------|---------|
| Methanol | 900 ml |
| Acetic acid | 200 ml |
| Distilled water to | 2000 ml |

3. Reagent for Blotting

3.1 Anode buffer NO I, 0.3 M Tris, pH 10.4

| | |
|-------------------------------------|----------|
| Tris (hydroxymethyl) - aminomethane | 36.33 ml |
| Methanol | 200 ml |
| Distilled water to | 1000 ml |
| Store at 4°C | |

3.2 Anode buffer No II, 25 mM Tris, pH 10.4

| | |
|-------------------------------------|-----------|
| Tris (hydroxymethyl) - aminomethane | 3.0274 ml |
| Methanol | 200 ml |
| Distilled water to | 1000 ml |
| Store at 4°C | |

3.3 Cathode buffer, pH 9.4

| | |
|-------------------------------------|-----------|
| Tris (hydroxymethyl) - aminomethane | 3.0274 ml |
| Aminocaproic acid | 13.248 ml |
| Methanol | 200 ml |
| Distilled water to | 1000 ml |
| Store at 4°C | |

4. Reagent for Immunostaining**4.1 Washing Buffer, pH 8.5**

| | |
|-----------------------------------|----------|
| Tris (hydroxymethyl) aminomethane | 6.060 gm |
| Sodium chloride | 8.725 gm |
| Sodium cyanide | 0.02 gm |
| Tween - 20 | 0.5 ml |
| Distilled water to | 1000 ml |

The pH was adjust to 8.5 and store at 4°C

4.2 Substrate diluent for Diaminobenzidine

| | |
|-----------------------------|----------|
| Sodium chloride | 4.625 gm |
| Disodium hydrogen phosphate | 1.425 gm |
| Sodium dihydrogen phosphate | 0.35 gm |
| Distilled water to | 1000 ml |

The pH was adjust to pH 7.4 and store at 4°C

4.3 Diaminobenzidine substrate (DAB),

| | |
|-------------------------|---------|
| Diaminobenzidine | 0.01 gm |
| Phosphate buffer saline | 30 ml |
| 30% Hydrogen peroxide | 10 ul |
| Freshly preparation | |

5. Reagent for Hemolytic Function Assay**5.1 Alsever's solution**

| | |
|--------------------|---------|
| Dextrose | 20.5 gm |
| Sodium citrate | 8.0 gm |
| Citric acid | 0.6 gm |
| Sodium chloride | 4.2 gm |
| Distilled water to | 1000 ml |

Dissolved the ingredients and mix. The solution was sterilized by membrane filtration and store at 4°C

5.2 Veronal buffer saline (VBS) - EDTA, pH 7.4

| | |
|-----------------------------|---------|
| Sodium chloride | 8.3 gm |
| Sodium barbital | 1.02 gm |
| EDTA solution, 0.2 M pH 7.4 | 50 ml |
| Distilled water to | 1000 ml |

The pH was adjust to pH 7.4 and store at 4°C

5.3 CaCl₂ solution, 30 mM

| | |
|--------------------|----------|
| Calcium chloride | 3.286 gm |
| Distilled water to | 500 ml |

5.4 Magnesium chloride solution, 100 mM

| | |
|--|-----------|
| Magnesium chloride (MgCl ₂ 6H ₂ O) | 10.165 gm |
| Distilled water to | 500 ml |

5.5 Veronal buffer saline (VBS), 5X, pH 7.4

| | |
|--------------------|----------|
| Sodium barbital | 3.75 gm |
| Barbitone | 5.75 gm |
| Sodium chloride | 85.00 gm |
| Distilled water to | 2000 ml |

The pH was adjust to pH 7.4 and store at 4°C

5.6 Gelatin-Veronal buffer saline (GVBS) + Mg²⁺ + Ca²⁺

| | |
|--------------------|---------|
| Gelatin | 1.0 gm |
| 5 X VBS | 200 ml |
| MgCl ₂ | 10 ml |
| CaCl ₂ | 5 ml |
| Distilled water to | 1000 ml |

5.7 Hydrazine sulfate solution, 0.15 M, pH 7.4

Hydrazine sulfate 1.95 gm

Distilled water to 100 ml

The pH was adjust to pH 7.4 and store at 4°C



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