

APPENDIX

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EQUIPMENTS AND CHEMICALS

1. Equipments

- 1.1 Autoclave: Tomy ES-315
- 1.2 Hot air oven: WTB BINDER
- 1.3 Incubator shaker: Innova™ 4340
- 1.4 Incubator: BINDER
- 1.5 Larmina flow: Astec Microflow ABS 1200
- 1.6 Mini-PROTEAN 3 Cell Electrophoresis: BIO-RAD
- 1.7 pH meter: BECKMAN
- 1.8 Refrigerated centrifuge: SORVALL RC50
- 1.9 Refrigerated centrifuge: Jouan MR22
- 1.10 Spectrophotometer: genesys 20
- 1.11 Transmission electron microscope: HITASHI H-700
- 1.12 Vortex mixer: Vortex-2 GENIE G-560E
- 1.13 Water bath: ISOTEMP 210

2. Chemicals

- 2.1 99.9% Acrylamide: BIO-RAD
- 2.2 Agar: DIFCO
- 2.3 Ammonium persulfate: USB Corporation
- 2.4 Bis-N, N'-Methylene-bis-acrylamide: BIO-RAD
- 2.5 Brain heart infusion: BD
- 2.6 di-Potassium hydrogen phosphate: UNIVAR
- 2.7 di-Sodium hydrogen phosphate: MERCK
- 2.8 Glycerol: CARLO-ERBA
- 2.9 Glycine: SIGMA
- 2.10 Magnesium chloride: CARLO-ERBA
- 2.11 2-Mercaptoethanol: SIGMA-ALDRICH

- 2.12 Methylene blue: Fluka
- 2.13 Potassium hydroxide: CARLO ERBA
- 2.14 Sodium dodecyl sulfate (SDS): CARLO-ERBA
- 2.15 Potassium dihydrogen phosphate: UNIVAR
- 2.16 Sodium dihydrogen phosphate: MERCK
- 2.17 Sodium chloride: CARLO ERBA
- 2.18 N, N, N', N'-Tetramethylethylenediamine (TEMED; USB Corporation)
- 2.19 Tris-hydroxymethyl-methylamine (Tris-base): UNIVAR
- 2.20 Triton-X 100: CARLO-ERBA
- 2.21 Tryptic Soy Broth: BD
- 2.22 Vancomycin: SIGMA-ALDRICH

3. Preparation

3.1 Brain heart infusion (BHI) (1000 ml)

Suspended 37 g of the powder in 1000 ml of distilled water.

Sterilized by autoclaving at 121°C for 15 minutes.

3.2 Tryptic soy broth (TSB) (1000 ml)

Suspended 30 g of the powder in 1000 ml of distilled water.

Sterilized by autoclaving at 121°C for 15 minutes.

3.3 0.1M Potassium phosphate buffer (K_2HPO_4 - KH_2PO_4), pH 7.0 (500 ml)

Added 30.75 ml of 0.1 M di-Potassium hydrogen phosphate.

Added 19.25 ml of 0.1 M Potassium dihydrogen phosphate.

Adjusted the pH to 7.0 and added distilled water to 500 ml.

Sterilize by autoclaving at 121°C for 15 minutes.

3.4 0.1M Sodium phosphate buffer (Na_2HPO_4 - NaH_2PO_4), pH 7.0 (500 ml)

Added 28.85 ml of 0.1 M di-Sodium hydrogen phosphate.

Added 21.15 ml of 0.1 M Sodium dihydrogen phosphate.

Adjusted the pH to 7.0 and added distilled water to 500 ml.

Sterilized by autoclaving at 121°C for 15 minutes.

3.5 1M Tris-HCl, pH 8.0 (500 ml)

Dissolved Tris-base 65.5 g in distilled water.

Adjusted the pH to 8.0 and added distilled water to 500 ml.

Sterilized by autoclaving at 121°C for 15 minutes.

3.6 Renaturation buffer (500 ml)

Added 3.25 ml of 1M Tris-HCl, pH 8.0.

Added 5 ml of Triton X-100.

Added 5 ml of 10mM Magnesium chloride.

Added distilled water to 500 ml.

3.7 Working solution for electrophoresis

3.7.1 30% (w/v) acrylamide solution (100 ml)

Dissolved 29.2 g of acrylamide in distilled water.

Dissolved 0.8 g of bis-acrylamide in distilled water.

Added distilled water to 100 ml.

3.7.2 4X separating gel buffer (100 ml)

Added 75 ml of 2M Tris-HCl, pH 8.8.

Added 4 ml of 10% (w/v) SDS.

Added distilled water to 100 ml.

3.7.3 4X stacking gel buffer (100 ml)

Added 50 ml of 1M Tris-HCl, pH 6.8.

Added 4 ml of 10% (w/v) SDS.

Added distilled water to 100 ml.

3.7.4 5X Tris-Glycine buffer, pH 8.3 (1000 ml)

Dissolved 15 g of Tris-base in distilled water.

Dissolved 72 g of Glycine in distilled water.

Dissolved 5 g of SDS in distilled water.

Adjusted pH the pH to 8.3 and added distilled water to 1000 ml.

3.8 Preparation of gel for electrophoresis

3.8.1 Separating gel (10% acrylamide; 10 ml)

Dissolved 0.01 g of lyophilized *Micrococcus luteus* cells in 4.2 ml of distilled water.

Added 3.3 ml of 30% (w/v) acrylamide solution

Added 2.5 ml of 4X separating gel buffer.

Added 50 μ l of 10% (w/v) ammonium persulfate.

Added 5 μ l of TEMED.

3.8.2 Stacking gel

Added 0.67 ml of 30% (w/v) acrylamide solution.

Added 1 ml of 4X stacking gel buffer.

Added 2.3 ml of distilled water.

Added 30 μ l of 10% (w/v) ammonium persulfate.

Added 5 μ l of TEMED.