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# APPENDICES

# APPENDIX A

1. **Reagents for plasmid preparation**

   **1.1. Lysis solution:** 10 mL

| | | |
|---|---|---|
| 50% Glucose | 2 | mL |
| 0.5 M EDTA | 0.2 | mL |
| 1 M Tris-HCl (pH 8.0) | 0.25 | mL |
| $ddH_2O$ | 7.55 | mL |

   **1.2. Alkaline SDS solution:** 10 mL

| | | |
|---|---|---|
| 5% SDS | 2 | mL |
| 5 N NaOH | 0.4 | mL |
| $ddH_2O$ | 7.6 | mL |

   **1.3. Hight salt solution:** 3 M Sodium acetate (pH 5.2)

| | | |
|---|---|---|
| $NaOAc.3H_2O$ | 408.1 | g |
| $ddH_2O$ | 700 | mL |

   Adjust pH to 5.2 with glacial acetic and adjust the volume to 1 litre with water.

2. **Other reagents for preparation**

   **2.1. RNase A solution**

   Dissolve RNase A (pancreatic) at a concentration of 10 mg/mL in 10 mM Tris-HCl (pH 7.5), 15 mM NaCl, then heat at 100 °C, 15 min, cool slowly at room temperature, aliquot, and store at -20 °C.

   **2.2. 10% Glycerol**

| | | |
|---|---|---|
| Glycerol | 10 | mL |
| Water | 90 | mL |

   **2.3. 5× TBE buffer** (for agarose gel electrophoresis)

| | | |
|---|---|---|
| Tris-base | 54 | g |
| Boric acid | 27.5 | g |
| 0.5 M EDTA (pH 8) | 20 | mL |

**2.4. 0.5 M EDTA**

| | | |
|---|---|---|
| EDTA | 186.1 | g |
| Water | 1000 | mL |

Dissolve EDTA in 800 mL water and adjust pH to 8.0 with NaOH before adjusting volume to 1 litre, and then autoclave.

**2.5. 1M Tris-HCl**

| | | |
|---|---|---|
| Tris-base | 121.1 | g |
| Water | 1000 | ml |

Adjust pH to 7-8 before adjusting volume to 1 litre, and then autoclave.

**2.6. 5% SDS** (store at room temperature)

| | | |
|---|---|---|
| SDS | 5 | g |
| Water | 100 | mL |

**2.7. 5N NaOH**

| | | |
|---|---|---|
| NaOH | 20 | g |
| Water | 100 | mL |

Dissolve NaOH in 70 ml water before adjusting volume to 100 mL

**2.8. Loading buffer** (for agarose gel electrophoresis)

| | | |
|---|---|---|
| Glycerol | 20 | mL |
| Bromphenol blue | 4 | mg |
| Water | 80 | mL |

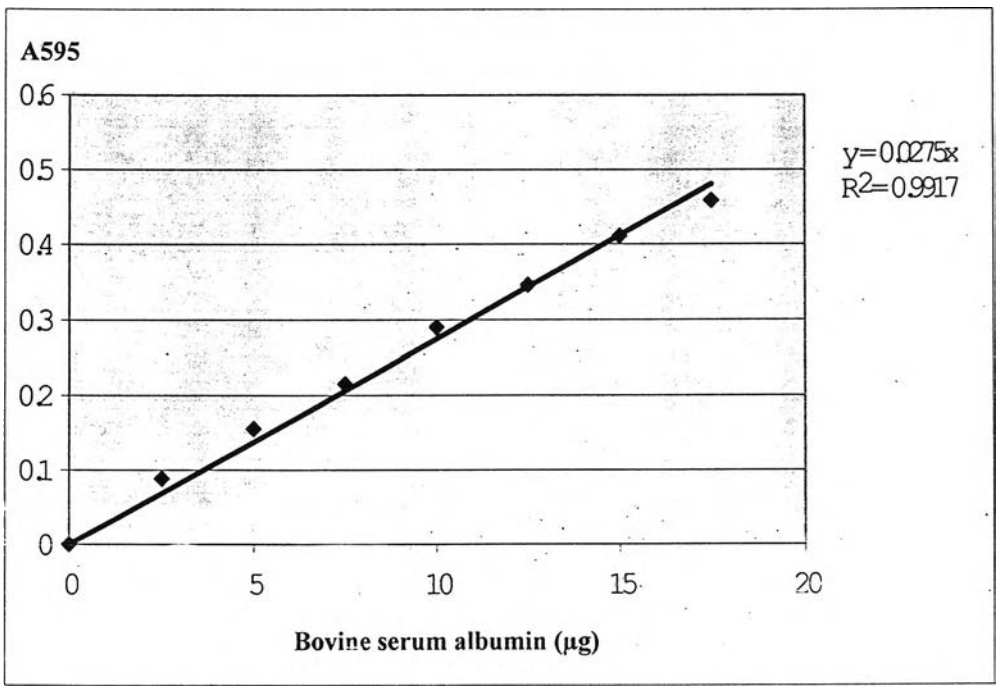
**2.9. Coomassie blue reagent**

| | | |
|---|---|---|
| Coomassie blue G 250 | 50 | mg |
| 95% Ethanol | 25 | mL |
| 85% $H_3PO_4$ | 50 | mL |

Adjust volume to 500 mL with water.

# APPENDIX B

Standard curve for protein determination by Coomassie blue method.



# BIOGRAPHY

Miss Raevadee Siritunyanont was born July 30, 1971. She graduated with the Bachelor Degree of Science in Biology from Srinakharinwirot University in 1994. She has worked Department of Medical Sciences, Ministry of Public Health. She has studied for Master of Science in Biochemistry Program, Faculty of Science, Chulalongkorn University since 2003.