

Appendix

1. Chemical stock solution and buffer

0.5 M Tris-HCl

60.55 g of Tris (hydroxymethyl) aminomethane was dissolved in 800 ml of distilled water. The pH was adjusted to 7.5 or the desire value by adding concentrated HCl. The volume of the solution was adjusted to 1000 ml with distilled water.

0.05 M Tris-HCl, pH 7.5 – 0.15 M NaCl (TBS)

0.5 M Tris-HCl, pH 7.5	50 ml
NaCl	4.38 g

The ingredients were dissolved in distilled water and the volume was adjusted to 500 ml.

0.05 M Tris-HCl, pH 7.5-0.3 M NaCl-0.1 M CaCl₂ (TB-NaCa)

0.5 M Tris-HCl, pH 7.5	50 ml
NaCl	8.76 g
CaCl ₂	5.55 g

The ingredients were dissolved in distilled water and the volume was adjusted to 500 ml.

Phosphate buffer saline (PBS)

Stock solution (0.1 M phosphate buffer-0.3 M NaCl)

KH ₂ PO ₄	2.6 g
Na ₂ HPO ₄ · 7H ₂ O	21.7 g
NaCl	17.42 g

The ingredients were dissolved in distilled water. The pH was adjusted to 7.4 with HCl. The solution was brought up to 1,000 ml with distilled water.

10% Ammonium persulphate (APS)

APS	0.01 g
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APS was dissolved in 1 ml of distilled water and stored at 4 °C.

10% SDS

SDS	10 g
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SDS was dissolved in 90 ml of distilled water and heated in hot water bath and adjusted the volume to 100 ml with distilled water. It was stored at room temperature.

2. Buffer for electrophoresis**Electrophoresis buffer for Native-PAGE**

Tris (hydroxymethyl) aminomethane	3.02 g
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Glycine	14.4 g
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The ingredients were dissolved in 1,000 ml of distilled water.

Electrophoresis buffer for SDS-PAGE

Tris (hydroxymethyl) aminomethane	3.02 g
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Glycine	14.4 g
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SDS	1 g
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The ingredients were dissolved in 1,000 ml of distilled water.

4X SDS gel loading buffer

0.2 M Tris-HCl, pH 6.8

0.008 M EDTA

40% glycerol

4% SDS

0.4% Bromophenol blue

1% β -mercaptoethanol (for reducing condition)

The volume of the solution was adjusted to 50 ml with distilled water and stored at 4 °C.

3. Dot blot and western blot buffer

Blocking buffer: TBS containing 3% BSA

Washing/assay buffer (TBST): TBS containing 0.05% Tween 20

Transfer blotting buffer:

Tris (hydroxymethyl) aminomethane	6.055 g
Glycine	28.82 g
20% methanol	

The ingredients were dissolved in 200 ml distilled water and stirred on a magnetic stirrer for several minutes to ensure that the ingredients have dissolved. 400 ml of methanol was added, dissolved and bring the solution up to 2,000 ml with distilled water.

4. Media for bacteria culture

PCA (Plate Count Agar)

Plate count agar	23.5 g
NaCl	15 g

The ingredients were dissolved in 1,000 ml of distilled water and sterilized by autoclaving for 15 minutes at 15 psi. The medium was poured into glass or plastic plate.

TCBS Agar

TCBS Agar	89.0 g
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The medium was suspended in 1,000 ml of distilled water and boiled to dissolve completely. It was cooled to 50 °C and poured into sterile petri plates.

TSB (Tryptic Soy Broth)

TSB Soybean-Casein Digest Medium	30.0 g
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NaCl	15 g
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The powder was suspended in 1,000 ml of distilled water and warmed slightly to dissolve the powder. The medium was sterilized by autoclaving for 121 °C for 15 minutes.

TSA (Tryptic Soy Agar)

TSB Soybean-Casein Digest Medium	30.0 g
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NaCl	15 g
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Agar	15 g
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The ingredients were dissolved in 1,000 ml of distilled water and sterilized by autoclaving at 15 psi for 15 minutes. The medium was poured into glass or plastic plate.

5. Calibration curve for protein determination

5.1 Calibration curve for protein concentration by Bradford

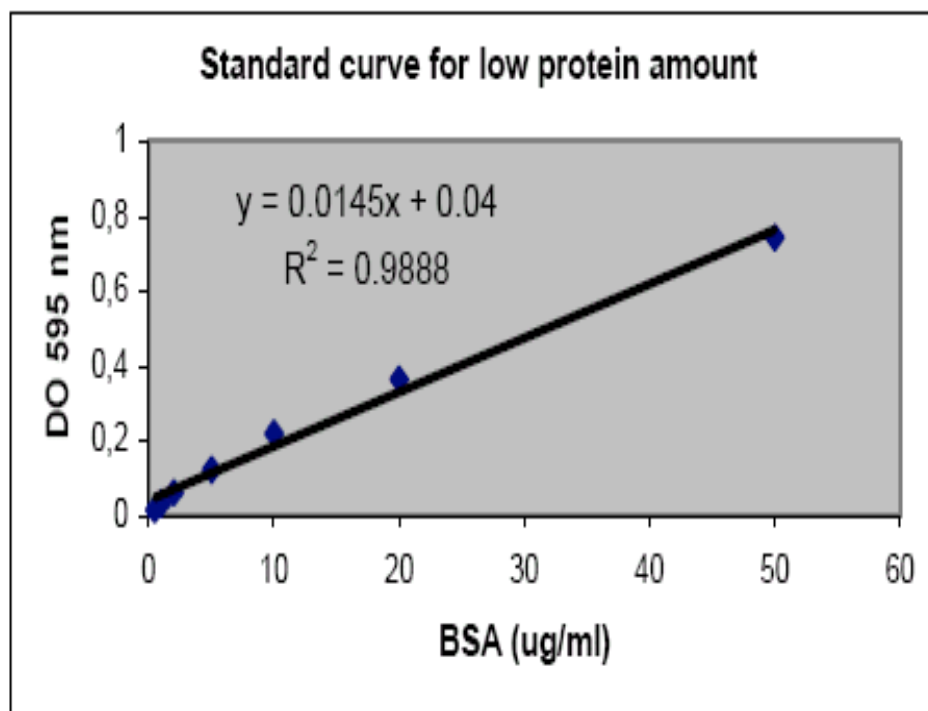


Fig. 43. Standard curve for protein concentration by Bradford (1976)

5.2 Calibration curve for protein concentration by Bicinchoninic acid (BCA)

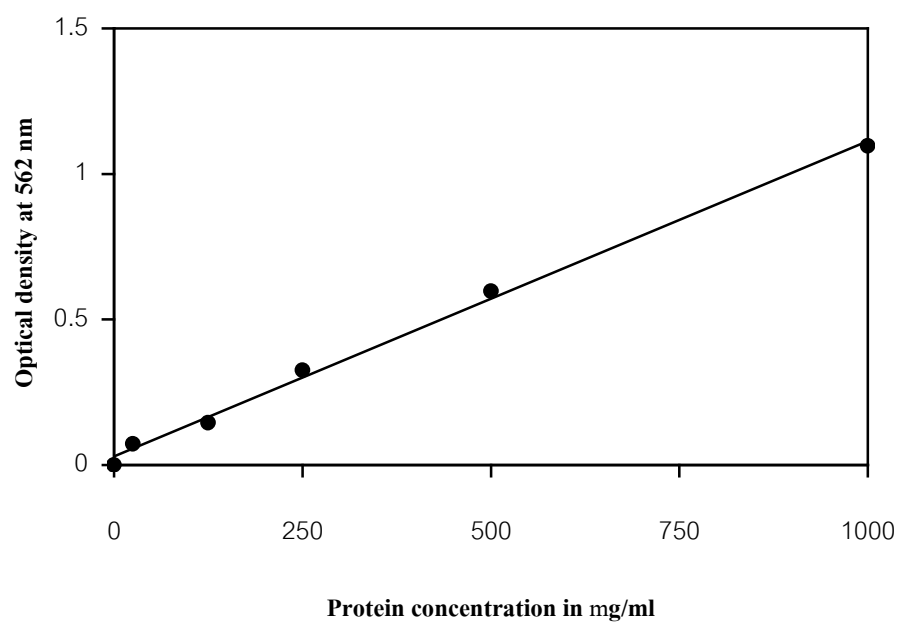


Fig. 44 Calibration curve for protein concentration by Bicinchoninic acid (BCA) method (Stoscheck, 1990)