9. APPENDIX
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a. 10× Ligase Buffer
   500 mM Tris-HCl (pH 7.5)
   70 mM MgCl₂
   10 mM dithiothreitol (DTT)

b. Column-Loading Dye
   50% (v/v) glycerol
   10% (v/v) 10× STE buffer
   40% (w/v) saturated BPB II
   To make saturated bromophenol blue (BPB), add a small amount of BPB crystals to water and vortex. Centrifuge the sample briefly and look for the presence of an orange pellet. If a pellet is seen, the solution is saturated. If not, add more crystals and repeat the procedure.

c. SM Buffer (per Liter)
   5.8 g of NaCl
   2.0 g of MgSO₄ · 7H₂O
   50.0 ml of 1 M Tris-HCl (pH 7.5)
   5.0 ml of 2% (w/v) gelatin
   Add deionized H₂O to a final volume of 1 liter

d. 20× SSC Buffer (per Liter)
   175.3 g of NaCl
   88.2 g of sodium citrate
   800.0 ml of deionized H₂O
   Adjust to pH 7.0 with a few drops of 10 N NaOH
   Add deionized H₂O to a final volume of 1 liter

e. 10× STE Buffer
   1 M NaCl
   200 mM Tris-HCl (pH 7.5)
   100 mM EDTA
   10× Alkaline Buffer (per 50 ml)
   3 ml of 5.0 M NaOH
   2 ml of 0.5 M EDTA
   45 ml of deionized H₂O
f. Formaldehyde Gel Loading Buffer
   720 µl of formamide
   160 µl of 10× MOPS buffer
   260 µl of 37% formaldehyde
   100 µl of sterile water
   100 µl of Ethidium bromide (10 mg/ml)
   80 µl of sterile glycerol
   80 µl of saturated BPB II in sterile water

g. 5% Non-denaturing Acrylamide Gel
   Mix the following in a vacuum flask
   5 ml of 10× TBE buffer
   8.33 ml of a 29:1 acrylamide–bis-acrylamide solution
   36.67 ml of sterile deionized H2O
   De-gas this mixture under vacuum for several minutes
   Add the following reagents
   25 µl of TEMED
   250 µl of 10% ammonium persulfate

h. Alkaline Agarose 2× Loading Buffer
   200 µl of glycerol
   750 µl of water
   46 µl of saturated BPB II
   5 µl of 5 M NaOH

i. 10× MOPS Buffer
   200 mM 3-[N-morpholino]propane-sulfonic acid (MOPS)
   50 mM sodium acetate
   10 mM EDTA
   Adjust to a final pH of 6.5–7.0 with NaOH
   Do not autoclave

j. 700 mM β-Mercaptoethanol
   5 µl of 14 M β-mercaptoethanol
   95 µl of DEPC-treated water

k. 1× TAE Buffer
   40 mM Tris-acetate
   1 mM EDTA
I. LB Broth (per Liter)

- 10 g of NaCl
- 10 g of tryptone
- 5 g of yeast extract

Add deionized H₂O to a final volume of 1 liter
Adjust to pH 7.0 with 5 N NaOH
Autoclave

II. LB Agar (per Liter)

- 10 g of NaCl
- 10 g of tryptone
- 5 g of yeast extract
- 20 g of agar

Add deionized H₂O to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH
Autoclave
Pour into petri dishes (~25 ml/100-mm plate)

III. LB-Kanamycin Agar (per Liter)

Prepare 1 liter of LB agar
Autoclave
Cool to 55°C
Add 6.6 ml of 7.5 mg/ml filter-sterilized kanamycin
Pour into petri dishes (~25 ml/100-mm plate)

IV. LB-Tetracycline Agar (per Liter)

Prepare 1 liter of LB agar
Autoclave
Cool to 55°C
Add 1.5 ml of 10 mg/ml filter-sterilized tetracycline
Pour into petri dishes (~25 ml/100-mm plate)
Store plates in a dark, cool place or cover plates with foil if left out at room temperature for extended time periods as tetracycline is light-sensitive

V. LB Broth with Supplements

Prepare 1 liter of LB broth
Autoclave
Add the following filter-sterilized supplements prior to use
- 10 ml of 1 M MgSO₄
- 3 ml of a 2 M maltose solution or 10 ml of 20% (w/v) maltose
q. LB Top Agar (per Liter)
   10 g of NaCl
   10 g of tryptone
   5 g of yeast extract
   Add 0.7% (w/v) agarose
   Adjust to pH 7.0 with 5 N NaOH
   Add deionized H₂O to a final volume of 1 liter
   Autoclave

r. LB–Ampicillin Agar (per Liter)
   1 liter of LB agar, autoclaved
   Cool to 55°C
   Add 10 ml of 10-mg/ml filter-sterilized ampicillin
   Pour into petri dishes: (~25 ml/100-mm plate)

s. Lysozyme Buffer
   50 mM Tris-HCl (pH 8.0)
   150 mM NaCl
   5 mM MgCl₂
   3% (w/v) BSA
   Add the following just before use:
   Lysozyme to 400 μg/ml
   DNase to 1 U/ml

t. SM Buffer
   100 mM NaCl
   50 mM Tris-HCl (pH 7.5)
   10 mM MgSO₄
a. Blocking Solution
   1% (w/v) BSA in TBS

b. Antibody Diluent
   1% (w/v) BSA in TBS

c. Tris-Buffered Saline (TBS)
   20 mM Tris-HCl (pH 7.5)
   150 mM NaCl

d. Tris-Buffered Saline containing Tween 20 (TBST)
   TBS
   0.05% (v/v) Tween 20

e. Color Development Solution
   100 mM Tris-HCl (pH 9.5)
   100 mM NaCl
   5 mM MgCl₂

f. Stop Solution
   20 mM Tris-HCl (pH 2.9)
   1 mM EDTA

II. LB Agar (per Liter)
   10 g of NaCl
   10 g of tryptone
   5 g of yeast extract
   20 g of agar
   Adjust pH to 7.0 with 5 N NaOH
   Add deionized H₂O to a final volume of 1 liter
   Autoclave
   Pour into petri dishes (~25 ml/100-mm plate)

LB—Ampicillin Agar (per Liter)
   1 liter of LB agar, autoclaved
   Cool to 55°C
   Add 10 ml of 10 mg/ml filter-sterilized ampicillin
   Pour into petri dishes (~25 ml/100-mm plate)
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i. LB Top Agar (per Liter)
   10 g of NaCl
   10 g of tryptone
   5 g of yeast extract
   Add 0.7% (w/v) agarose
   Adjust to pH 7.0 with 5 N NaOH
   Add deionized H$_2$O to a final volume of 1 liter
   Autoclave

j. Lysozyme Buffer
   50 mM Tris-HCl (pH 8.0)
   150 mM NaCl
   5 mM MgCl$_2$
   3% (w/v) BSA
   Add the following just before use:
   Lysozyme to 400 µg/ml
   DNase to 1 U/ml

k. NZY Agar (per Liter)
   5 g of NaCl
   2 g of MgSO$_4$.7H$_2$O
   5 g of yeast extract
   10 g of NZ amine (casein hydrolysate)
   15 g of agar
   Adjust the pH to 7.5 with NaOH and autoclave
   Pour into petri dishes (~80 ml/150-mm plate)

NZY Top Agar (per Liter)
   5 g of NaCl
   2 g of MgSO$_4$.7H$_2$O
   5 g of yeast extract
   10 g of NZ amine (casein hydrolysate)
   Add 0.7% (w/v) agarose
   Adjust the pH to 7.5 with NaOH
   Add deionized H$_2$O to a final volume of 1 liter and autoclave

SM Buffer
   100 mM NaCl
   50 mM Tris-HCl (pH 7.5)
   10 mM MgSO$_4$
A. Buffers and solutions for indirect haemagglutination test

Phosphate buffer saline pH6.4

- NaH₂PO₄ : 4.5 gms
- Na₂HPO₄ : 2.13 gms
- NaCl : 3.07 gms
- Distilled water : 700 ml

Phosphate buffer saline pH 7.2

- NaH₂PO₄ : 1.8 gms
- Na₂HPO₄ : 5.3 gms
- NaCl : 3.1 gms
- Distilled water : 700 ml

Others

- Bovine serum albumin (0.1%) : BSA 1 gm in 100 ml of PBS pH 7.2
- NaCl in distilled water (1.7%) : 1.7 gms of NaCl in 100 ml of distilled water
- Na₂CO₃ in distilled water (1%) : 1 gm of Na₂CO₃ in 100 ml of distilled water
- Aqueous pyruvic aldehyde (40% v/v):
- Aqueous glutaraldehyde (2% v/v): in PBS pH 7.2

Isever’s solution

- Dextrose : 2.05 gms
- Sodium citrate : 800 mg
- Sodium chloride : 420 mg
- Citric acid : 5.5 mg
- Distilled water : 100 ml

1% tannic acid in PBS pH 7.2 (1:25,000) (Always Freshly prepared)

- Tannic acid : 10 mg
- PBS pH 7.2 : 250 ml

Renssen phosphate buffer pH 7.2

1 of 15 M NaH₂PO₄ (9.078 gms/1 litre H₂O) + 3 ml of 15 M KH₂PO₄ (11.86 gms/1 litre H₂O)

Reagents for estimation of proteins by Lowry’s method

(i) 2% Sodium potassium tartarate – 2 gms of sodium potassium tartarate in 100 ml distilled water
APPENDIX

(ii) 1% Copper Sulphate - 1gm of copper sulphate in 100ml distilled water.

(iii) 2% Sodium Carbonate solution in 0.1N NaOH (0.4gm / 100ml double distilled water) - 2gms of sodium carbonate dissolved in 0.4gm of sodium hydroxide solution.

(iv) Alkaline Copper reagent - 1 ml each of reagents (i) and (ii) mixed together at the time of experiment and made upto 100ml with reagent (iii)

(v) 1N folin's reagent.

C. Reagents and solutions for SDS-PAGE

Stock solutions

- 2M Tris-HCl (pH 8.8) - 100ml
- 1M Tris-HCl (pH 6.8) - 100ml
- 10% (w/v) SDS - store at room temperature
- 50% (v/v) Glycerol - 100ml
- 1% (w/v) Bromophenol blue - 10ml (solution filtered after preparation to remove aggregated dye)
- TEMED-(N,N,N',N'-tetramethylene-ethylenediamine)
- 2-mercaptoethanol or, Dithiothreitol
- Glycine

Working solutions:

- Acrylamide stock (30%) - 100ml
  - Acrylamide 29.2 g
  - Bisacrylamide 0.8 g
  - Add distilled water to make 100ml & stir until completely dissolved
- Separating Gel Buffer (4x) - 100ml
  - 75ml 2M Tris-Hcl (pH 8.8) 1.5 M
  - 4ml 10% SDS 0.4 %
  - 21ml distilled water
- Stacking Gel Buffer (4x) - 100ml
  - 50ml 1M Tris-Hcl (pH 6.8) 0.5 M
  - 4ml 10% SDS 0.4 %
  - 46ml distilled water
  - 10% Ammonium persulfate (APS) 5ml
  - 0.5g APS dissolved in 5ml distilled water
- Electrophoresis / Running Buffer (1x) - 1000ml
  - 3g Tris 25 mM
  - 14.4g Glycine 192 mM
  - 1g SDS 0.1 %
  - Distilled water to make upto 1000 ml
- Sample Buffer - 10ml
  - 0.6ml 1M Tris-Hcl (pH 6.8)-60mM
  - 5ml 50% Glycerol 25 %
  - 2ml 10% SDS 2 %
  - 0.5ml 2-mercaptoethanol 14.4 mM
  - 1ml 1% Bromophenol blue 0.1 %
  - 0.9ml Distilled water
- Staining solution - 1000ml
  - 1.0g Coomassie Blue R-250
  - 45ml Methanol

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- 450ml Distilled water
- 100ml Glacial acetic acid
- Destaining solution-1000ml
  - 100ml Methanol
  - 100ml Glacial acetic acid
  - 800ml Distilled water

Sample preparation: The sample antigen preparation (5-10μl) is mixed with sample buffer (10-20μl) in an eppendorf tube and heated at 75-100°C for 2-10 minutes.

Different concentrations of gel: 12% and 10% for separation gel and 5% for stacking gel

<table>
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<th>Reagent</th>
<th>10% gel</th>
<th>12% gel</th>
<th>Reagents</th>
<th>5% gel</th>
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<tr>
<td>Acrylamide stock</td>
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<td>4 ml</td>
<td>Acrylamide stock</td>
<td>1.6 ml</td>
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<tr>
<td>Total volume</td>
<td>10 ml</td>
<td>10 ml</td>
<td>Total volume</td>
<td>10 ml</td>
</tr>
</tbody>
</table>

D. Reagents and buffers for EITB

Transfer buffer (pH between 8.1-8.4 without adjustment)

- Tris 3.0 g
- Glycine 14.5 g
- Methanol 200 ml.
- Distilled H₂O make up to 1000 ml.

Phosphate buffered saline (pH 7.2)

- Na₂HPO₄ 5.76 gm.
- NaH₂PO₄ 1.5 gm.
- NaCl 9.0 gm.

(Make the volume to 1000 ml.)

Blocking solution: 3 % BSA in PBS 7.2

Substrate buffer (PBS-T): PBS 7.2 containing 0.1 % Tween-20

Substrate solution for Horse radish peroxidase

- Diaminobenzidine(DAB) 6 mg.
- Substrate buffer 10 ml.
- 20 % H₂O₂ 10 μl.

XLV
Antibody / Conjugate dilution buffer

- (PBS-T + BSA) = 0.5 % BSA in PBS-T

Washing buffer (PBS-T) : PBS 7.2 containing 0.1% Tween-20 (1 ml. of Tween-20 in 1 litre of PBS )

E. Buffers for ELISA

Coating buffer : carbonate bicarbonate buffer pH 9.6

- Na₂CO₃ : 1.5 gms
- Na₂HCO₃ : 2.93 gms
- The salts are dissolved in 950 ml of distilled water. The pH is adjusted to 9.6 by adding 1N HCl/1N NaOH. The volume is made upto 1000 ml with distilled water.

Phosphate buffered saline pH 7.2 used in ELISA is prepared as mentioned earlier.

The washing buffer and substrate/conjugate dilution buffer are as mentioned for the EITB.