1. BACTERIAL CULTURE MEDIA AND REAGENTS:

1.1. Luria Bertani (LB) Broth

   Tryptone  10 g  
   Yeast Extract  5 g  
   NaCl  5 g  

   The components were dissolved in 950 ml Milli RO water and the pH adjusted to 7.0 with 5 N NaOH and the volume adjusted to 1000 ml with Milli RO water.

1.2. LB Agar (solid media)

   For preparing solid media, 2% agar was used to the LB broth. Medium was sterilized by autoclaving.

1.3. Antibiotic

   Ampicillin (100 mg/ml) antibiotic solutions was prepared and filter sterilized by a 0.22 µm filter (Millipore) and stored at -20 °C for long-term use.

1.4. Reagents for competent cells preparation

   Solution I:

   CaCl2  0.1 M  
   Stored at 4°C

   Solution II:

   CaCl2  0.1 M  
   Glycerol  15%  
   Stored at 4°C

   All the reagents and buffers for DNA and Bacterial culture work were prepared in Milli Q water and sterilized by autoclaving for 20 minutes at 15-psi pressure.

2. COMMONLY USED BUFFERS & REAGENTS

2.1. Common Buffers

   Phosphate Buffered Saline (PBS), per liter (pH 7.4)

   KH2PO4  0.24 g  
   Na2HPO4  1.44 g  
   NaCl  8.0 g  
   KCl  0.2 g
**Tris-HCl buffer (pH 8.0)**

Tris-HCl buffer was prepared by dissolving appropriate amount of Tris base in distilled water and adjusting the pH with 2N HCl to 8 and stored at RT.

**Ethylene diamine tetra acetic acid (EDTA)**

0.5 M solution of disodium salt of EDTA was prepared in distilled water, pH adjusted to 8.0 with 10 N NaOH Solution and stored at RT.

**Tris-Buffered Saline (TBS) pH 7.4 for 1 litre**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris-base</td>
<td>3.0 gm</td>
</tr>
<tr>
<td>NaCl</td>
<td>8.0 gm</td>
</tr>
<tr>
<td>KCl</td>
<td>0.2 gm</td>
</tr>
</tbody>
</table>

pH was adjusted to 7.4 with 2N HCl.

**2.2. Reagents for Agarose Electrophoresis**

**TAE Buffer (50X) for 1 liter**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris Base</td>
<td>242 g</td>
</tr>
<tr>
<td>Glacial Acetic Acid</td>
<td>57.1 ml</td>
</tr>
<tr>
<td>0.5 M EDTA (pH 8.0)</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

Stored at RT

**TE Buffer**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris-HCl (pH 8.0)</td>
<td>10 mM</td>
</tr>
<tr>
<td>EDTA</td>
<td>1 mM</td>
</tr>
</tbody>
</table>

**6X BPB dye for agarose gel electrophoresis**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromophenol Blue</td>
<td>0.25 %</td>
</tr>
<tr>
<td>Sucrose</td>
<td>40 %</td>
</tr>
</tbody>
</table>

Stored at 4°C

**2.3. Reagents for SDS-Polyacrylamide gel electrophoresis**

**Acrylamide (30%) for 100 ml**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylamide</td>
<td>29.0 g</td>
</tr>
<tr>
<td>N, N’-methylenebisacrylamide</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Final volume</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

The solution was filtered through Whatman filter paper No. 1 and stored at 4°C.

**Ammonium per sulfate (APS) 10%**

Prepared fresh, 0.1 g of APS in 1 ml of Milli Q water.

**SDS (10%)**

10 g SDS in 100 ml Milli Q water and stored at RT
**Buffer for resolving gel**
1.5 M Tris-HCl (pH 8.8), stored at RT.

**Buffer for stacking gel**
1.0 M Tris-HCl (pH 6.8), stored at RT.

**Tris-glycine Running buffer (Laemmli buffer), per liter pH 8.3**
- Tris Base 3.0 g
- Glycine 14.4 g
- SDS 1.0 g

**Laemmli sample buffer (5X)**
- Tris (pH 6.8) 60 mM
- Glycerol 25 %
- SDS 2 %
- β-mercaptoethanol 14.4%
- Bromophenol Blue 0.1 %

Final volume adjusted with TDW and stored at -20ºC.

**Staining solution (Coomassie blue)**
- Coomassie brilliant blue (R250) 0.25 %
- Acetic Acid 10 %
- Methanol 45 %
- Milli RO water 45%

The solution was filtered through Whatman filter paper No. 1 and stored at RT.

**Destaining solution**
- Methanol 45%
- Acetic acid 10%
- Milli RO water 45%

Stored at RT.

2.4. Reagents for Western Blot analysis

**Transfer buffer, per liter**
- Tris-base 2.42 g
- Glycine 11.26 g
- Methanol 100 ml

Always prepared fresh.

**Ponceau S staining solution**
- Ponceau S 0.5 g
Glacial acetic acid 1 ml
Milli Q water 99 ml
Stored at RT.

**Blocking Buffer (100ml)**

- Skimmed milk 5% (w/v)
- Tween-20 0.1% (v/v)

Always prepared fresh in 1X PBS.

**Washing Buffer (PBST)**

1X PBS containing 0.1% Tween-20 and always prepared fresh.

**Developing Solution**

- Diaminobenzidine (DAB) 10 mg
- H$_2$O$_2$ 6 µl
- Imidazole 10 mg

Prepared in 1X PBS

### 2.5. Reagents for protein purification

All the buffers prepared fresh and filtered through 0.45 µm filter.

**Sodium Phosphate buffer (pH 6.5) 1M (100 ml)**

- NaH$_2$PO$_4$ (1M) 68.5 ml
- Na$_2$HPO$_4$ (1M) 31.5 ml

**Lysis buffer (pH 7.0)**

- NaH$_2$PO$_4$ 50 mM
- NaCl 600 mM
- Imidazole 10 mM
- Glycerol 10% (v/v)

**Wash buffers (pH 7.0)**

- NaH$_2$PO$_4$ 50 mM
- NaCl 600 mM
- Imidazole 50 mM
- Glycerol 10% (v/v)
- Triton X-100 0.1% (v/v)
- β-mercaptoethanol 1 mM

**Elution buffer (pH 7.0)**

- NaH$_2$PO$_4$ 50 mM
- NaCl 300 mM
Imidazole  250 mM

*Dialysis buffer (pH 7.0)*

NaH$_2$PO$_4$  50 mM
NaCl  200 mM

*Buffer for Gel filtration chromatography*

1M Sodium Phosphate Buffer (pH -6.5) 50 mM
NaCl  200 mM

2.6. Reagents used for ELISA

*Coat buffer (Sodium carbonate buffer, pH 9.6) 100 ml*

NaHCO$_3$  0.293 g
Na$_2$CO$_3$  0.159 g

*Blocking buffer*

1 % BSA in 1X PBST

*Assay diluent*

0.25% BSA in 1X PBS

*Citrate buffer*

(A) Citric acid  1.92 g in 100ml Milli Q
(B) Na$_2$HPO$_4$  2.84 g in 100ml Milli Q

Fresh prepared citrate buffer (pH 5.0) with 12.2 ml of solution (A) and 12.8 ml of solution (B) for 25 ml buffer.

*Ortho phenylene diamine (OPD) substrate solution*

OPD  20 mg
Citrate buffers (pH-5.0) 25 ml
H$_2$O$_2$  20 µl

*Stop solution (2.5N H$_2$SO$_4$) 10 ml*

H$_2$SO$_4$  2.2 ml
TDW  8.8 ml
List of Publication:

