APPENDIX A

1. Protein concentration determination

The concentration of protein was determined by Lowly’s method. Protein standard BSA was diluted in distilled water to concentrations of 0.2, 0.5, 0.8, 1.2, 1.5 mg/ml. A volume of 100 ul of either protein standard or protein samples was mixed with 4.5 ml of Lowly’s reagent (Bio-Rad). The absorbance at 750 nm was determined from protein standard. The concentration of protein sample was calculated by referring to the concentration of protein standard.

2. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

The method of SDS-PAGE was performed as described by Laemmli, 1970. The gel solution was prepared as shown in Table 4. Electrophoresis was carried out in the descending direction on the Tris-glycine buffer (25 mM Tris-HCl, pH 6.8, 192 mM glycine and 0.1% (w/v) SDS) using a constant 100 V for 30 min and 200 V for 2 h or until the tracking dye reached the edge of the gel.
### Table 4. Preparation of SDS-Polyacrylamide gel

<table>
<thead>
<tr>
<th>Solution</th>
<th>4% Stacking gel</th>
<th>12% Resolution gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1.88 ml</td>
<td>3.23 ml</td>
</tr>
<tr>
<td>40% Acrylamide(acrylamide: $N,N'$-methylenebisacrylamide, 29:1)</td>
<td>0.3 ml</td>
<td>2.1 ml</td>
</tr>
<tr>
<td>1.5 M Tris-HCl, pH 8.8</td>
<td>-</td>
<td>1.52 ml</td>
</tr>
<tr>
<td>0.5 M Tris-HCl, pH 6.8</td>
<td>0.75 ml</td>
<td>-</td>
</tr>
<tr>
<td>10% SDS</td>
<td>0.03 ml</td>
<td>0.07 ml</td>
</tr>
<tr>
<td>10% APS</td>
<td>0.03 ml</td>
<td>0.07 ml</td>
</tr>
<tr>
<td>TEMED</td>
<td>0.01 ml</td>
<td>0.01 ml</td>
</tr>
</tbody>
</table>

**SDS** = Sodium Dodecyl Sulfate  
**APS** = Ammonium Persulfate  
**TEMED** = $N,N,N',N'$-tetramethylenediamine
APPENDIX B

1. Chemical stock solution and buffer

5 M Tris-HCl

- Tris-base: 6 g
- Distilled water: 100 ml

Adjust the pH to 8.8 by adding concentrated HCl. Adjust the volume of the solution to 100 ml with distilled water and sterilize by autoclaving.

5 M Tris-HCl

- Tris-base: 6 g
- Distilled water: 100 ml

Adjust the pH to 6.8 by adding concentrated HCl. Adjust the volume of the solution to 100 ml with distilled water and sterilize by autoclaving.

10% SDS (w/v):

- SDS: 10 g
- Distilled water: 90 ml

Heat to 68 °C and stir with a magnetic stirrer to assist dissolution. Adjust the volume to 100 ml with distilled water and store at room temperature. Sterilization is not necessary.

0.1% APS (w/v):

- Ammonium persulfate: 1 g
- Distilled water: 10 ml
**Phosphate-buffered Saline (PBS):1X**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>8 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.2 g</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>1.44 g</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.24 g</td>
</tr>
</tbody>
</table>

Dissolve the ingredients in 800 ml of distilled water. Adjust the pH to 7.4 with HCl. Add distilled water to 1000 ml. Sterilize the buffer by autoclaving and store at room temperature or 4 °C

**Ponceau S**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ponceau S</td>
<td>10 g</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>50 ml</td>
</tr>
<tr>
<td>Deionized water to</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

**0.5% Trypsin**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypsin</td>
<td>0.5 g</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.02 g</td>
</tr>
<tr>
<td>1xPBS</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

**SDS lysis buffer**

- 2% (w/v) SDS
- 50 mM Tris, pH6.8
- 10% glycerol
2. Solutions for electrophoresis

Tris-glycine buffer, Running buffer: 10X

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris base</td>
<td>30.28 g</td>
</tr>
<tr>
<td>Glycine</td>
<td>144.13 g</td>
</tr>
<tr>
<td>SDS</td>
<td>10 g</td>
</tr>
</tbody>
</table>

Dissolve the ingredients in 800 ml of distilled water. Adjust the volume to 1000 ml with distilled water.

SDS Gel loading buffer: 4X

- 200 mM Tris-HCl, pH 6.8
- 8% (w/v) SDS
- 0.4% (w/v) bromophenol blue
- 40% (v/v) glycerol
- 8% (v/v) 2-B-mercaptoethanol
- 400 mM DTT

Adjust the volume of the solution to 50 ml with distilled water and store at -80°C.

Comassie blue staining

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comassie Brilliant Blue R250</td>
<td>0.025 g</td>
</tr>
<tr>
<td>Methanol</td>
<td>43 ml</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>7 ml</td>
</tr>
<tr>
<td>Distilled water to</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

Destain solution

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>30 ml</td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>7 ml</td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>63 ml</td>
<td></td>
</tr>
</tbody>
</table>
3. Solution for Western blot analysis

Electroblotting buffer: 1X

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris-base</td>
<td>5.8 g</td>
</tr>
<tr>
<td>Glycine</td>
<td>7.9 g</td>
</tr>
<tr>
<td>Methanol</td>
<td>200 ml</td>
</tr>
</tbody>
</table>

Dissolve the ingredients in distilled water and bring up to volume 1000 ml with distilled water.

TBS-T buffer: 5X

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tween®20</td>
<td>5 ml</td>
</tr>
<tr>
<td>NaCl</td>
<td>45 g</td>
</tr>
<tr>
<td>Tris-HCl</td>
<td>31.75 g</td>
</tr>
<tr>
<td>Tris-base</td>
<td>5.8 g</td>
</tr>
</tbody>
</table>

Dissolve the ingredients in distilled water and bring up to volume 1000 ml with distilled water.

Blocking buffer:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low fat dry milk</td>
<td>5 g</td>
</tr>
<tr>
<td>1X TBS-T</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

Washing buffer:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low fat dry milk</td>
<td>10 g</td>
</tr>
<tr>
<td>1XTBS-T</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

Stripping buffer

100 mM β-mercaptoethanol (14.3 M stock)

2% (w/v) SDS 10 ml

62.5 mM Tris-HCl, pH 6.8

Incubate the membrane at 60 °C 30 min and then wash with TBS-T, 2x10 min.