## LPS PAGE with Silver Stain

### Materials:
**Lysis buffer**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>In 1L</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS</td>
<td>2g</td>
</tr>
<tr>
<td>2-Mercaptoethanol</td>
<td>4mL</td>
</tr>
<tr>
<td>Bromophenol Blue</td>
<td>0.003g</td>
</tr>
<tr>
<td>1M tris-Cl pH6.8</td>
<td>to 100mL</td>
</tr>
</tbody>
</table>

**SDS-PAGE gel**

<table>
<thead>
<tr>
<th></th>
<th>10% separating gel</th>
<th>4% stacking gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.3mL 30% acrylamide/0.8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>bisacrylamide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.5mL water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.75mL 4x Tris-Cl/SDS pH 8.8</td>
<td>1.25mL 4x Tris-Cl/SDS pH 6.8</td>
<td></td>
</tr>
<tr>
<td>2.88g Urea</td>
<td>25μL 10%APS</td>
<td></td>
</tr>
<tr>
<td>50μL 10%APS</td>
<td>5μ TEMED</td>
<td></td>
</tr>
<tr>
<td>10μL TEMED</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Fixation Solution:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>for 200mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>isopropyl alcohol</td>
<td>50mL</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>14mL</td>
</tr>
<tr>
<td>ddH2O</td>
<td>up to 200mL</td>
</tr>
</tbody>
</table>

### Oxidation Solution:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>for 150mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periodic acid</td>
<td>1.05g</td>
</tr>
<tr>
<td>EtO&lt;sub&gt;h&lt;/sub&gt;</td>
<td>4mL</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>500μL</td>
</tr>
<tr>
<td>ddH2O</td>
<td>146mL</td>
</tr>
</tbody>
</table>
**Staining Reagent**

-on a 50mL Falcon tube

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1M NaOH</td>
<td>2mL</td>
</tr>
<tr>
<td>Ammonium hydroxide</td>
<td>2.6mL</td>
</tr>
<tr>
<td>ddH2O</td>
<td>41mL</td>
</tr>
</tbody>
</table>

-on a 15mL Falcon tube

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>AgNO3</td>
<td>1g</td>
</tr>
<tr>
<td>ddH2O</td>
<td>5mL</td>
</tr>
</tbody>
</table>

**Developing Solution**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% citric acid</td>
<td>2mL</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>100μL</td>
</tr>
<tr>
<td>ddH2O</td>
<td>200mL</td>
</tr>
</tbody>
</table>

**Stop Solution**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic Acid</td>
<td>1.6mL</td>
</tr>
<tr>
<td>ddH2O</td>
<td>up to 200mL</td>
</tr>
</tbody>
</table>

**Methods:**

**Preparing the samples:**
1. Resuspend cells on PBS.
2. Make a 1 mL 1:100 aliquot of the cells.
3. Determine OD at 600nm.
4. Calculate \((5/\text{OD})_{1000} = \text{amount of cells needed for 1mL 5OD resuspension.}\)
5. Spin down at 8krpm for 5 min
6. Resuspend in 200μL Lysis buffer by pipetting up and down.
7. Boil for 10 mins.
8. Add 3 μL 20 μg/μL Proteinase K; incubate at 60C for 1h.

**Making the gel:**
1. Install equipment.
2. Mix the reagents for the separating gel.
3. Pour separating gel, cover with a layer of ddH2O; wait for 45 mins or until gel polymerizes. Take out water layer.
4. Mix reagents for stacking gel, and pour. Avoid bubbles.
5. Insert comb. Allow to polymerize for 45 mins or until gel polymerizes.
6. Install gel in the equipment. Add 1x SDS running buffer.
7. Load 6μL of Kaleidoskope Marker, and 20 of each sample. Run at 35mAmp for 2h.
**LPS Silver stain:**

1. Place the gel in fixation solution ON at 4C
2. Replace with oxidation solution for 5 mins with gentle agitation
3. Wash gel in 200μL ddH2O for 30 mins, repeat 3 times. Change to a clean glass dish after rinses.
4. Mix the two components of the staining reagent (pour silver nitrate slnt into the 50mL falcon tube SLOWLY; a brown ppt will for briefly, but dissolves quickly). Remove the last wash and add fresh staining reagent. Shake for 10 minutes)
5. Wash the gel in ddH2O for 10 mins X4.
6. Add developing soln for 10-20 mins, The LPS bands appear slowly until yellow background staining of gel appears.
7. Stop development by washing on Stop solution.
8. Rinse the gel in ddH2O, store in the dark at 4C.