Protocol: Ponceau Stain

Application:
Staining protein on PVDF or nitrocellulose membranes post-transfer (i.e. Western blotting) for the purpose of protein transfer verification.

Recipe:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O</td>
<td>950 mL</td>
<td>XXX</td>
</tr>
<tr>
<td>Glacial Acetic Acid*</td>
<td>50 mL</td>
<td>5% vol/vol</td>
</tr>
<tr>
<td>Ponceau Red dye</td>
<td>1 gram</td>
<td>0.1% wt/vol</td>
</tr>
</tbody>
</table>

**Total volume of solution** **1000 mL**

*Caution: *Open glacial acetic acid in chemical fume hood.*
**Store final solution at 4 °C.

Procedure:

1. Post-transfer, place membrane into flat-bottom container (protein side up).

2. Completely submerge with Ponceau stain and put on rocker
   - Nitrocellulose membranes saturate in ~5 min.
   - PVDF membranes saturate in ~15 minutes.

3. Remove Ponceau from non-protein parts of the membrane via gentle rinsing with distilled H₂O.

4. Capture image to verify blot quality.

5. Mark molecular weight standards and lanes to configure blot using *permanent* lab marker.

6. Completely remove Ponceau with 4-5x room temperature washes (~5-10 minutes per wash)
   - Using 1x TBS/0.2% Tween-20.

7. Once all the “red” is gone, membrane can be blocked and probed as per normal procedure.