Protein quantification using BCA protein assay kit (Pierce)

1. Prepare standard (25-400 µg/ml) using the following procedure:
   a. Add 12 µl 2mg/ml BSA (standard come with the kit) to 48 µl of H₂O
   b. Make serial dilution (take 30 µl to 30 µl of H₂O) to make the following standard.
2. Add 25 µl of each standard or unknown sample into a microplate well.
3. Add 200 µl of the working solution (mix part A and B in 50:1 ratio) to each well and mix.
4. Shield from light and incubate at 37°C for 30 min.
5. Determine O.D. 569nm using microplate reader.

Following is one example of OD₅₉₆nm:

<table>
<thead>
<tr>
<th>400 µg/ml</th>
<th>200 µg/ml</th>
<th>100 µg/ml</th>
<th>50 µg/ml</th>
<th>25 µg/ml</th>
<th>0 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.900</td>
<td>0.460</td>
<td>0.233</td>
<td>0.139</td>
<td>0.93</td>
<td>0.088</td>
</tr>
</tbody>
</table>