ELISA Protocol

1. Coat antigen (5 µg/ml, diluted by 1 x PBS) into each well with 150 µl volume.
   What kind of plate?

2. Cover the plate and incubate at 4C overnight.

3. Decant by flicking the plate and wash with 4% BSA/1 x PBS for 10 mins. Repeat 3 times.

4. Fill the wells with 150 µl diluted serum, ascites or sample containing (dilution depends on the sample), cover and incubate at room temperature for 1h.

5. Flick the plate and wash with 4 % BSA/1 x PBS for 10 mins., repeat two times.

6. Fill the well with anti-mouse or human IgG conjugated with alkaline phosphatase (AP, normally diluted 1/1000~1/2500 by 1 x PBS), cover and incubate at room temperature for 1h.

7. Flick the plate and wash three times with 4 % BSA/1 x PBS (10 mins. each).

8. Make substrate solution with AP Base Buffer and Phosphatase Substrate Tablets (Sigma, No.104-105, final conc. 1 mg/ml).

9. Fill the well with 150 µl of substrate solution and read the color development by Microplate reader at 405 nm.

10. If it is necessary to save the ELISA plate, the color can be preserved by adding 100 µl of 1 N NaOH to each well. Cover the plate and store it in the freezer.

**AP Base Buffer**

<table>
<thead>
<tr>
<th>Volume</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>96 ml</td>
<td>di-ethanol amine</td>
</tr>
<tr>
<td>1180 µl</td>
<td>2 M MgCl₂</td>
</tr>
<tr>
<td>800 ml</td>
<td>H₂O</td>
</tr>
</tbody>
</table>

adjust to pH 9.8
finally mess up to 1L