Using a NanoDrop™ to Measure Antibody Concentration

If an antibody sample is free of protein-based carriers (e.g. BSA, gelatin) or certain interfering preservatives such as thimerosal, a simple and non-destructive scan of the IgG sample on a NanoDrop spectrophotometer can be used to determine antibody concentration. This saves the trouble of performing a Bradford protein assay to confirm the initial antibody concentration. To determine antibody concentration using a NanoDrop spectrophotometer, follow these steps (the exact procedure may vary based on your NanoDrop model):

1. Turn on the NanoDrop spectrophotometer and click on the NanoDrop icon to launch the software.

2. Place a 2 µL drop of ultrapure water on the clean pedestal, then click OK (if required).

3. When the main menu appears, select the Protein A280 menu option.

   **Note:** Do not use the UV-Vis menu option on the NanoDrop to read an antibody sample.

4. After the Protein A280 menu appears, de-select the 340 nm normalization option by un-checking the corresponding box.

   **Note:** Some instruments do not have this normalization feature, in which case this step can be ignored.

5. In the window labeled Sample Type, select ‘Other Protein E1%’ from the pull-down menu. Enter the appropriate E1% value (see Table 1) corresponding to your particular antibody type. For example, the E1% for mouse IgG is 14.00.

6. Blank the NanoDrop spectrophotometer by placing a 2 µL drop of the appropriate sample buffer (e.g. PBS) on the pedestal and click the ‘Blank’ icon.

7. Immediately click on the ‘Measure’ icon to ensure there is a flat baseline. Clean the pedestal and repeat (if necessary) until a flat baseline is obtained.

   **Note:** Sometimes air bubbles can become trapped on the pedestal during sample application which can cause baseline offsets. If necessary, remove air bubbles and rescan to ensure a proper baseline.

8. Place a 2 µL aliquot of antibody solution on the clean pedestal and click the ‘Measure’ icon. Wait until the spectrum (220-350 nm) appears in the window. Note: For precious or limited samples, the majority of the 2 µL aliquot can be recovered from the pedestal.

9. Record the antibody concentration (mg/mL) directly from the NanoDrop display window. Alternatively, calculate the antibody concentration manually as illustrated below.

**Example:** Mouse IgG at 1 mg/mL in PBS, pH 7.2 was scanned as described and its concentration confirmed using equation #1.

![NanoDrop spectrum](image)

**Equation #1:**

\[
\text{[A}_{280}/E1\%\text{ value}] \times 10 \text{ mg/mL} = \text{protein concentration (mg/mL)}
\]

Example: Mouse IgG @ 1 mg/mL

10 mm pathlength A280 reading (from scan) = 1.34

Antibody E1% value (from Table 1) = 14.00

\[
[1.34 / 14.00] \times 10 \text{ mg/mL} = 0.96 \text{ mg/mL}
\]

<table>
<thead>
<tr>
<th>Antibody Source</th>
<th>Antibody E1% (1-cm path)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human IgG</td>
<td>13.60</td>
</tr>
<tr>
<td>Human IgE</td>
<td>15.30</td>
</tr>
<tr>
<td>Rabbit IgG</td>
<td>13.50</td>
</tr>
<tr>
<td>Donkey IgG</td>
<td>15.00</td>
</tr>
<tr>
<td>Horse IgG</td>
<td>15.00</td>
</tr>
<tr>
<td>Mouse IgG</td>
<td>14.00</td>
</tr>
<tr>
<td>Rat IgG</td>
<td>14.00</td>
</tr>
<tr>
<td>Bovine IgG</td>
<td>12.40</td>
</tr>
<tr>
<td>Goat IgG</td>
<td>13.60</td>
</tr>
</tbody>
</table>

Mass extinction coefficients (E1%) used for calculating antibody concentrations. The E1% is the absorbance at 280 nm of a 10 mg/mL solution in a 1-cm pathlength cuvette.