

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) 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.

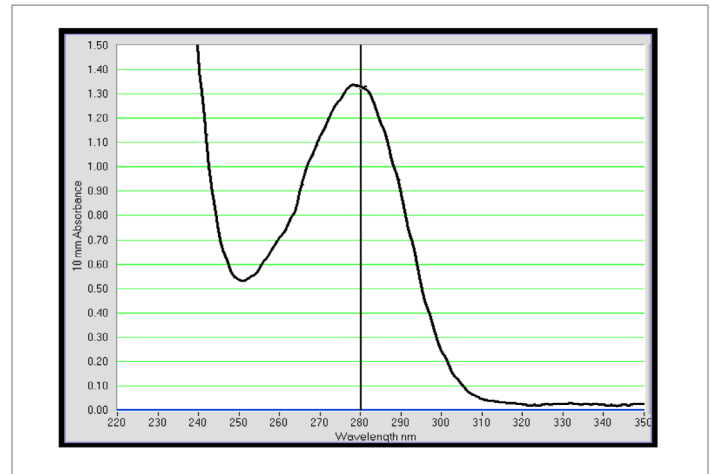
Note: Sometimes air bubbles can become trapped on the pedestal during sample application which can cause baseline offsets. If necessary, remove air bubbles and rescan to ensure a proper baseline.

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.



Equation #1:

$$[A_{280} / E1\% \text{ value}] \times 10 \text{ mg/ml} = \text{protein concentration (mg/ml)}$$

Example: Mouse IgG at 1 mg/ml

10 mm pathlength A₂₈₀ reading (from scan) = 1.34

Antibody E1% value (from Table 1) = 14.00

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

Antibody Source	Anttbody E1% (1-cm path)
Human IgG	13.60
Human IgE	15.30
Rabbit IgG	13.50
Donkey IgG	15.00
Horse IgG	15.00
Mouse IgG	14.00
Rat IgG	14.00
Bovine IgG	12.40
Goat IgG	13.60

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.