

Influence of Buffer on Protein Quantification at 280 nm

The most common protein quantification methods include direct absorbance at 280 nm, colorimetric assays, and fluorescence assays. The choice of quantification method depends on several factors, including the approximate protein concentration and whether it has been purified. A frequently overlooked factor that should be taken into consideration when choosing a protein quantification method is the buffer in which the protein is suspended.

The absorbance spectra of most protein buffers and components show some absorbance in the lower UV region (fig. 1), likely caused by various salts present in the buffer. This absorbance, however, typically decreases to baseline by ~230 nm. These buffers therefore do not affect the accuracy of A₂₈₀ quantification. Notable exceptions to this included RIPA, NDSB, and Triton X-100 buffers, which do possess significant absorbances at 280 nm (fig. 1).

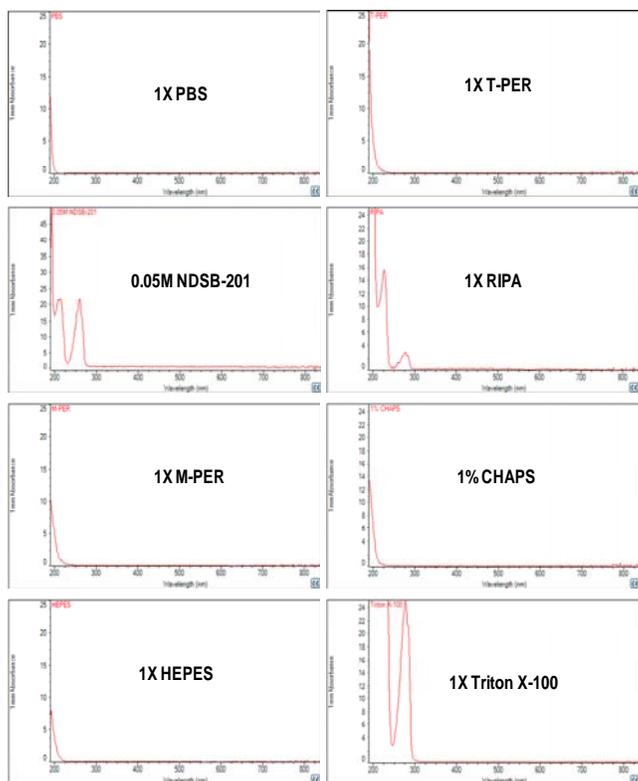


Figure 1: Absorbance of various buffers and buffer components (instrument blanked using deionized water). Spectra obtained using the NanoDrop 2000

The large absorbance at 280 nm of the RIPA buffer is

most likely due to its NP-40 or Triton X-100 content. Surfactants such as these strongly absorb light in the UV region at or near the analysis wavelength. Similarly, the ring structure in the NDSB molecule likely accounts for the strong absorbance in the UV regions demonstrated with this buffer.

The use of buffers with significant absorbance at 280 nm may result in quantification errors when making direct 280 nm measurements. When these buffers are used as a blanking solution for direct 280 nm measurements, after accounting for the large 280 nm absorbance, any spectrophotometer, including Thermo Scientific NanoDrop Spectrophotometers, could either underestimate or give negative final results. *It is good practice, however, to always blank using the buffer in which a sample is suspended.*

The effect of a strongly absorbing buffer can be seen in fig. 2 on the next page. This shows the absorbance of BSA samples in 0.5x PBS or 0.5x RIPA buffers. A clear deviation between the two spectra was observed across the monitored wavelength range, even though each protein sample was measured against a blank of the appropriate buffer. In this case, the use of a RIPA buffer resulted in a greater than 20% error in concentration measurement and compromised measurement precision. As a result, it is generally recommended that A₂₈₀ measurements not be performed using a buffer that exhibits significant absorbance at or near the analysis wavelength.

By measuring the buffer in which a protein is suspended against a pure, deionized water blank, the absorbance profile of the buffer can be observed. The amount of absorbance at 280 nm can then help determine whether the buffer is suitable for protein quantification by direct A₂₈₀ measurements.

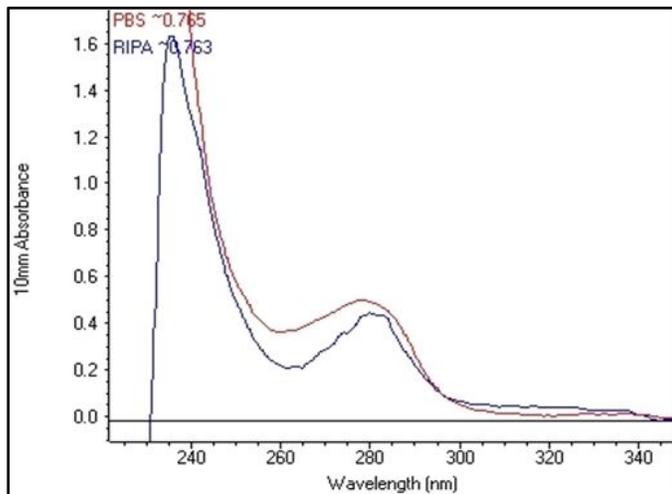


Figure 2: Absorbance spectra of 0.76 mg/ml protein solutions in PBS (red) and RIPA buffer (blue). RIPA buffer resulted in a greater than 20% error in concentration measurement and compromised measurement precision. Spectra obtained using the NanoDrop 2000.

The steps below outline a protocol for determining the acceptability of a particular buffer when making using the NanoDrop™ Lite for protein measurements.

1. Ensure both the lower and upper pedestal measurement surfaces are clean.
2. Select “Protein” from the main menu. Select the assay type most similar to the protein to be measured.
3. Load a 2 μ L aliquot of dH₂O onto the lower measurement pedestal and lower the sampling arm.
4. Select **Blank**. After the measurement is complete, use a dry, lint-free lab wipe to remove the water from both the top and bottom measurement surfaces.
5. Repeat steps 3 and 4 with a fresh aliquot of dH₂O when prompted to confirm the “**Blank**.”
6. Load a 2 μ L aliquot of the protein buffer in question. Lower the arm, then press “**Measure**”

The result should be an absorbance value of no more than 0.04 A (10 mm absorbance equivalent) at 280 nm. If not, consider using a colorimetric method to quantify the protein samples.

For Technical Support, contact us at 877-724-7690, US & Canada, or worldwide at 302-479-7707, or send an email to nanodrop.techsupport@thermofisher.com.