Application Note 52647

Pierce 660 nm Micro-Volume Assay Advantages

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Introduction

Colorimetric protein assays are routinely used in life science laboratories as a means to quickly quantitate protein concentration. Thermo Scientific™ NanoDrop™ spectrophotometers can be easily used to perform such colorimetric assays. A clear advantage of NanoDrop instruments is that they interface with intuitive software that contains pre-configured modules for these assays, thus making the determination of protein concentration easy and time effective for today's researcher. The Coomassie Blue G-250 dye binding assay (Bradford protein assay[1]) has long been the method of choice for many life scientists to quantify protein concentration because of its convenience and ease of use.[2]

While Bradford assays can be performed on NanoDrop spectrophotometers, certain characteristics of this assay can lead to inconsistent results if careful techniques and micro-volume considerations are not taken into account while performing the assay. The following characteristics make the Bradford assay particularly susceptible to variability, especially when making micro-volume measurements:

- **Stable Endpoint –** Many formulations of the Bradford reagent do not have a stable endpoint and color formation continues to occur after the incubation period.
- **Formation of Dye Aggregates –** The Bradford reagent is known for forming protein-dye and dye-dye aggregates which can interfere with UV-Vis measurements.

- **Non-Linear Standard Curves –** The Bradford assay is prone to non-linear standard curves, which can lead to inaccuracies when determining protein concentration.
- **Effects on Pedestal Surface –** The presence of phosphoric acid and methanol in the Bradford reagent reduces the hydrophobicity of the pedestals. This can create problems during liquid column formation between the pedestals.

The recently launched Thermo Scientific™ Pierce™ 660 nm protein assay eliminates many of the Bradford assay issues that can occur, including problems associated with micro-volume measurements. The Pierce 660 nm Protein Assay has several advantages, including a simple format, a stable endpoint, a greater linear dynamic range, and a decreased likelihood to form dye-dye aggregates in protein samples.

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Comparison of Color Formation

The color formation that occurs during a protein assay takes place over time. Most colorimetric assays require an incubation step which allows the color reagent to bind to proteins with a subsequent shift in the reagent's absorbance spectrum.[3] An important feature of a colorimetric protein reagent is that it should have a stable endpoint, thus allowing color formation to reach a plateau. The data presented here shows that the Bradford reagent has a less stable endpoint than the Pierce 660 nm assay. Figure 1 compares protein-dye color formation reactions for both assays. The Pierce 660 nm assay quickly reaches a stable endpoint in the first 5–10 minutes. However, with the Bradford assay, it takes approximately 15 minutes for the rate of color formation to decrease and there continues to be significant increase in absorbance between 15 and 35 minutes. This data confirms that careful consideration must be taken with respect to the incubation periods of the protein standards and unknown samples when carrying out a Bradford assay.

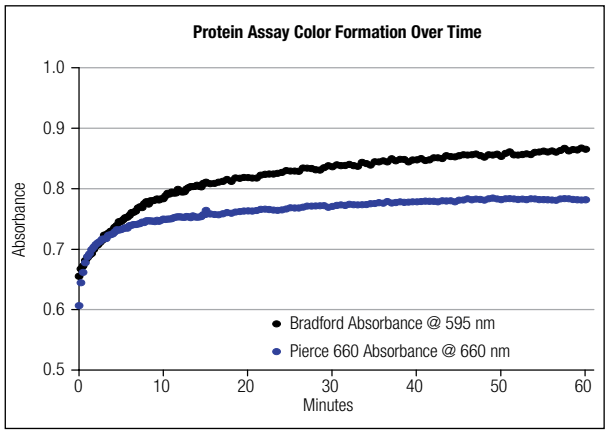

Figure 1: Absorbance change was monitored over time for both the Pierce 660 nm Protein assay and the Bradford assay. The Pierce 660 nm protein assay was monitored at 660 nm; the Bradford assay was monitored at 595 nm. Both reactions used the recommended protein to dye ratio (Pierce 660 nm used a reagent to protein ratio of 15:1, Bradford used a reagent to protein ratio of 50:1). The protein sample used was BSA in PBS at a concentration of 750 µg/mL for both reactions. Absorbance vs Time was monitored in a 10 mm cuvette using the NanoDrop 2000c kinetics module.

Formation of Dye Aggregates

Many Bradford reagent formulations have the tendency to form protein-dye and dye-dye aggregates as the reaction progresses. These aggregates can cause light scattering to occur. When these aggregates form in a particular reaction, its absorbance value will greatly deviate from the rest of the replicates, thus increasing the standard deviation from the average protein concentration. This has been found to be a common problem for all liquid Bradford dye reagents. The dye aggregates require dispersal before measurements are taken. Since these aggregates form relatively quickly, it is best to mix (vortex for 2–3 seconds) each tube or plate just before taking a micro-volume Bradford assay measurements.

Linear Dynamic Range

The Pierce 660 nm assay has a wide dynamic linear range when used with NanoDrop spectrophotometers. Protein concentrations ranges from 25 µg/mL to 2000 µg/mL can be measured with this assay depending on the sample to reagent ratio used. The Bradford assay high protein concentration specification is approximately 1000 µg/mL. For the comparison study illustrated in Figure 2, BSA standard curves for the Pierce 660 nm and Bradford assays were generated on a NanoDrop 8000 spectrophotometer. The Bradford standard curve shows its typical nonlinear response which is a result of the absorption spectra overlap of the bound and free dye.[2] A second order polynomial was used to fit the points of the Bradford assay whereas the 660 nm assay data was linear. Results demonstrate that the Pierce assay has a significantly greater linear dynamic range than the Bradford assay.

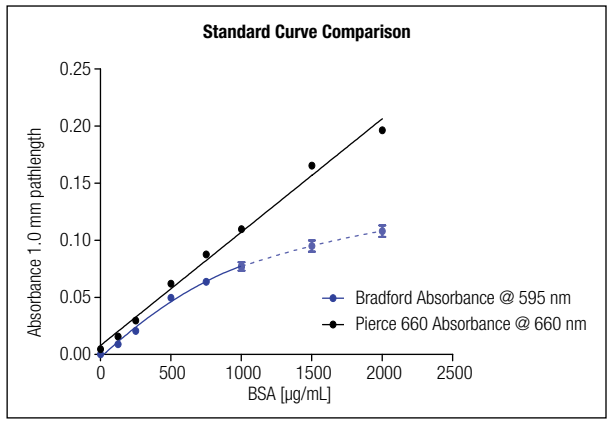

Figure 2: Pierce pre-diluted BSA standards were used to generate concentration response curves for both the pierce 660 nm assay and Bradford assay on the NanoDrop 8000. The reagent to sample ratio used for the Pierce 660 nm protein assay was 15:1 while the reagent to sample ratio used for the Bradford assay was 50:1.

Bradford Assay Effects on Pedestal Surface

NanoDrop spectrophotometers use a patented sample retention technology, which utilizes the hydrophobic properties of the optical pedestals along with the surface tension of the liquid sample to form a liquid column between two optical fibers. Formation of this liquid column is critical for accurate absorbance measurements. Some reagents used in life science have the tendency to reduce the hydrophobic properties of the optical pedestals making column formation more difficult. The combination of phosphoric acid and methanol present in the Bradford reagent formulations reduces the hydrophobicity of the pedestals with repeated use. Pedestal Reconditioning Compound (PR-1) is available from Thermo Fisher Scientific and can be used to restore the pedestal's hydrophobic nature. Conversely, the reagent used in the Pierce 660 nm assay does not un-condition the optical pedestals to the same degree as the Bradford reagent, thus decreasing the frequency of column breakage events.

Conclusion

Studies have shown that the Pierce 660 nm protein assay in conjunction with Thermo Scientific NanoDrop UV-Vis instruments is a more robust protein assay with a greater linear range than Bradford assays. This assay is well suited for the micro-volume samples used by the NanoDrop sample retention technology. The Pierce 660 nm protein assay has all the characteristics which are important for a robust colorimetric protein assay: easy to use format, a stable endpoint, and a broad dynamic linear range. The use of this assay may eliminate many of the drawbacks often encountered with the use of the Bradford assay.

References

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