Application Note: 51839

Total Protein Quantitation by the Thermo Scientific Pierce 660 nm Protein Assay

Nicole Kreuziger Keppy, Thermo Fisher Scientific, Madison, WI, USA

Key Words

- Bovine Gamma Globulin (BGG)
- Bovine Serum Albumin (BSA)
- Dye-binding Assays
- Linearity
- Local Control Software
- Total Protein Quantitation

Introduction

Total protein quantitation is a common measurement in life science, biopharmaceutical and food and beverage laboratories. Some of the most frequently used assays for total protein quantitation are dye binding assays, such as the Coomassie-based Bradford Assay. Although it is widely used, the Bradford Assay has several disadvantages. Many substances, including some detergents, flavonoids and protein buffers are known to interfere with the colorimetric properties this method relies on. Additionally, the linearity of the Bradford Assay is limited in both quality and range.

The broad substance compatibility and improved linearity of the Thermo Scientific Pierce 660 nm Assay in combination with its simple, single-reagent format make it a more accurate and convenient method for many routine applications. When performed on a Thermo Scientific UV-Visible spectrophotometer with embedded BioTest software, such as the Evolution™ 60S, BioMate™ 3S or GENESYS™ 10S Bio, the local control interface eliminates the need for manual programming of the Pierce™ 660 nm Protein Assay.

Background

The Thermo Scientific Pierce 660 nm Protein Assay is a quick, ready-to-use colorimetric method for total protein quantitation. The assay is reproducible, rapid and more linear than the Bradford Assay. In addition, the Pierce 660 nm Protein Assay is compatible with high concentrations of most commonly used reagents, such as detergents and reducing agents. As in a Bradford Assay, protein concentrations are estimated by reference to a series of standard protein dilutions assayed alongside the unknown samples.

Every total protein assay method exhibits some degree of varying response towards different proteins. These differences are related to variations in amino acid sequence, isoelectric point, structure and the presence of certain side chains or prosthetic groups that can dramatically alter the protein's color response. Thus, the selection of an appropriate reference protein is the key to obtaining high quality results.

The ideal protein to use as a reference standard in any protein assay is a purified preparation of the same protein that is to be measured in the sample. In the absence of such a reference protein, an alternative protein that produces a similar color response to that of the sample protein may be used.

The two most commonly used substitutes for a reference protein are Bovine Serum Albumin (BSA) and Bovine Gamma Globulin (BGG). BSA is a suitable standard when the sample contains primarily albumin, or the sample protein has a similar response to the dye as BSA. For a color response that is typical of purified antibodies and most non-serum protein mixtures (e.g., cell lysates), BGG is an appropriate standard protein.

About the Pierce 660 nm Assay

The Pierce 660 nm Protein Assay uses a proprietary dye-metal complex which binds to protein in acidic conditions, causing a shift in the dye's absorption maximum, which is measured at 660 nm. The dye-metal complex is reddish-brown in color, and turns green upon protein binding. This color change is produced by deprotonation of the dye at low pH facilitated by protein binding interactions between positively charged amino acid groups in the protein and the negatively charged deprotonated dye-metal complex (Figure 1).[1] Consequently, the complex interacts primarily with basic residues in the protein, such as histidine, arginine, and lysine; and, to a lesser extent tyrosine, tryptophan and phenylalanine.

The Pierce 660 nm Protein Assay has a protein-to protein variation of 37%; however, it is more linear then the Bradford assay (Figure 1).[2] Thus, it produces more accurate results when the appropriate standard is used. The linear detection range for the test tube method of the 660 assay is 25–2,000 µg/mL for BSA and 50–2,000 µg/mL for BGG.

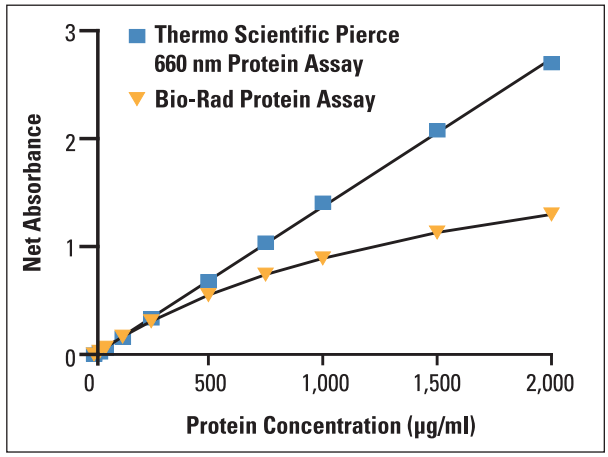

Figure 1: Performance comparison of the Thermo Scientific Pierce 660 nm Protein Assay vs. the Bio-Rad® Bradford Protein Assay. Both assays were performed according to the test-tube procedure using 100 µL of BSA. The Pierce 660 nm Protein Assay has a greater linear range (25–2,000 µg) compared with the Bradford Assay (125–1,000 µg). Absorbance values were measured at 660 nm for the 660 nm Protein Assay and 595 nm for the Bradford Protein Assay.

In addition, the color produced in the assay is stable and increases in proportion to a broad range of increasing protein concentrations, even in the presence of detergents and reducing agents that would be incompatible with Bradford and BCA Protein Assays.

The optional Ionic Detergent Compatibility Reagent (IDCR) may be added to the assay reagent to increase compatibility with high amounts of ionic detergents. This allows samples containing Laemmli SDS sample buffer with bromophenol blue to be measured. The IDCR completely dissolves by thorough mixing and does not have any affect on the assay. A list of maximal concentrations for substances known to be compatible with the Pierce 660 nm Protein Assay can be found in the product's instructions (Figure 2).

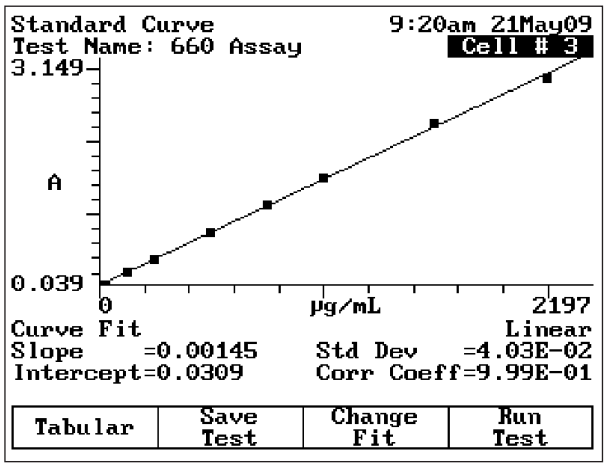

Figure 2: Example of a Pierce 660 Protein Assay BSA Standard Curve obtained on a Thermo Scientific GENESYS 10S UV-Visible spectrophotometer

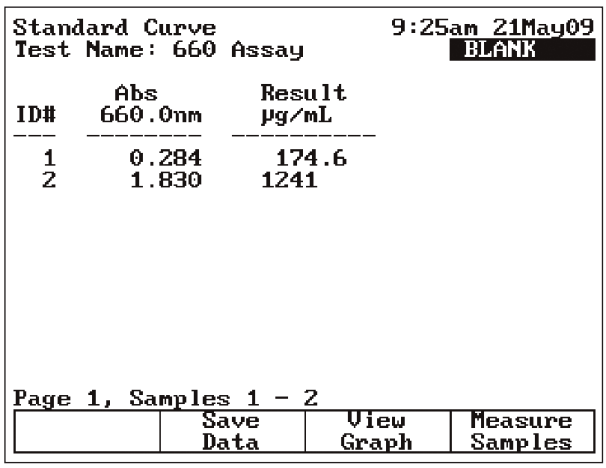

Figure 3: Example of a Pierce 660 Assay BSA Sample results obtained on a Thermo Scientific GENESYS 10S UV-Visible spectrophotometer

Experimental Method and Results

The Pierce Pre-diluted Protein Assay Standards: Bovine Serum Albumin (BSA) Set was used to prepare a standard curve. The kit contains seven standardized BSA solutions at concentrations of 125, 250, 500, 750, 1000, 1500 and 2000 µg/mL. A 25 µg/mL standard was prepared by mixing 10 µL of the 1000 µg/mL BSA standard with 390 µL of a prepared 0.9% saline and 0.05% sodium azide buffer solution. Two BSA samples of unknown concentration were also prepared using the 0.9% saline and 0.05% sodium azide buffer solution, and the buffer solution was used as the blank.

Following the provided 660 Assay instructions, 1.5 mL of assay reagent was added to 0.1 mL of each standard, sample and blank solution and incubated for five minutes. All solutions were read at a wavelength of 660 nm on a Thermo Scientific GENESYS 10S UV-Vis spectrophotometer with a 1.8 nm spectral bandwidth. The standard results, as shown in Figure 2, depict a linear curve fit with a correlation coefficient of 0.999, a clear indication that the Pierce 660 nm Protein Assay is linear for concentration of 25–2000 µg/mL. The sample results, as they are displayed in the local control software, are shown in Figure 3.

Performing the 660 Assay using Local Control BioTest Software

Embedded BioTest software in the local control interface of the Evolution 60S, BioMate 3S and GENESYS 10S Bio spectrophotometers provides pre-programmed life science assays, which includes the Pierce 660 nm Protein Assay and eliminates the need for manual programming. When using one of these instruments, simply select Protein Assays from the BioTest menu, then select the Pierce 660 nm Protein Assay from the list of assays shown in Figure 4 and follow the prompts.

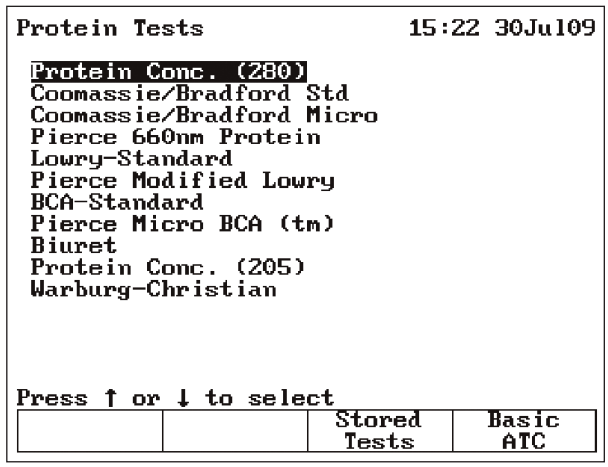

Figure 4: Example of the Protein Assay Menu screen on a Thermo Scientific BioMate 3S UV-Visible spectrophotometer

Summary

As we can see from this data, the Pierce 660 nm Protein Assay provides an easy-to-use, accurate and reliable method for total protein concentration measurements. In addition, combining the assay with a Thermo Scientific UV-Visible spectrophotometer with built-in BioTest software, such as the Evolution 60S, BioMate 3S and GENESYS 10S Bio, eliminates the need for manually programming the method. This simplifies the analytical process even further, saving time and reducing the potential for analyst errors.

References

1. U.S. Patent Application 20090197348, Pierce Biotechnology, Inc., 2009.
2. Pierce 660 nm Protein Assay Instructions, Thermo Fisher Scientific, 2008.

Ordering Information

Recommended systems and reagents for total protein analysis.

Description	Part Number
BioMate 3S UV-Vis, US line cord	840-208300
BioMate 3S UV-Vis, Europlug & UK line cords	840-209900
GENESYS 10S Bio UV-Vis, US line cord	840-207700
GENESYS 10S Bio UV-Vis, Europlug & UK line cords	840-209300
Evolution 60S UV-Vis, US line cord	840-208500
Evolution 60S UV-Vis, Europlug & UK line cords	840-210100
Pierce 660 nm Protein Assay Kit	22662

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