Determine acceptable wavelengths for measuring protein assays

TR0025.3

Introduction

Thermo Scientific® Pierce BCA, Coomassie (Bradford) and 660 nm Protein Assay methods involve development of colored products in the presence of protein. When samples do not contain substances that interfere with the specific reagent or dye-binding interaction, the intensity of the colored product is proportional to the amount of protein in the sample. Absorbance maxima for the colored products in these Pierce® BCA, Coomassie, and 660 nm Assays are 562 nm, 595 nm and 660 nm respectively. Typical spectrophotometers can be set to measure at these optimal wavelengths in the visible spectrum, but many microplate readers are equipped with filter sets for specific wavelengths that differ from the absorbance maxima required by the protein assay. In such situations, it is important to know the range of wavelengths that can be used and still obtain acceptable results.

The absorbance spectra presented in this Tech Tip provide a simple guide for determining which wavelengths are likely to be successful for measuring these protein assay results. The general response patterns observed in these data are representative of all variants of BCA and coomassie (Bradford) protein assay methods (see the following Product Information section) but were tested only for the two products indicated.

Each assay was performed using the standard test tube protocol and a set of pre-diluted bovine serum albumin (BSA) protein assay standards. Samples were prepared and incubated with protein assay reagent in glass test tubes and then transferred to 1 cm disposable cuvettes for measurement. Protein assay absorbances were measured in a spectrophotometer set to record at 5 nm increments across the specified wavelength range. Data are plotted in two different ways in the figures on the following pages: by wavelength (thereby producing a scan for each protein concentration) and by protein concentration (thereby producing a standard curve for each of several selected wavelengths).

Thermo Scientific Pierce Protein Assay Product Information

Current versions of this and other Tech Tips are available from the Technical Resources portion of our web site. Current product instructions and a complete listing of products are also available.

23225, 23227	**BCA Protein Assay Kit**, working range 20-2000 µg/ml
23235	**Micro BCA Protein Assay Kit**, working range 0.5-20 µg/ml
23250	**BCA Protein Assay Kit – Reducing Agent Compatible**, working range 125-2000 µg/ml
23236	**Coomassie Plus (Bradford) Assay Kit**, working range 1-1500 µg/ml
23238	**Coomassie (Bradford) Protein Assay Kit**, working range 1-1500 µg/ml
22660, 22662	**Pierce 660 nm Protein Assay,** working range 25-2000 µg/ml
23215	**Compat-Able™ Protein Assay Preparation Reagent Set**, sufficient reagents to pre-treat 500 samples to remove interfering substances before total protein quantitation
23209	**Bovine Serum Albumin Standard Ampules, 2 mg/ml**, 10 × 1 ml ampules, containing bovine serum albumin (BSA) at 2.0 mg/ml in 0.9% saline and 0.05% sodium azide
23208	**Bovine Serum Albumin Standard Pre-Diluted Set**, 7 × 3.5 ml aliquots in the range of 125-2000 µg/ml
23212	**Bovine Gamma Globulin Standard Ampules, 2 mg/ml**, 10 x 1 ml ampules
23213	**Bovine Gamma Globulin Standard Pre-Diluted Set**, 7 × 3.5 ml aliquots in the range of 125-2000 µg/ml

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BCA Protein Assay (Product No. 23225, 23227)

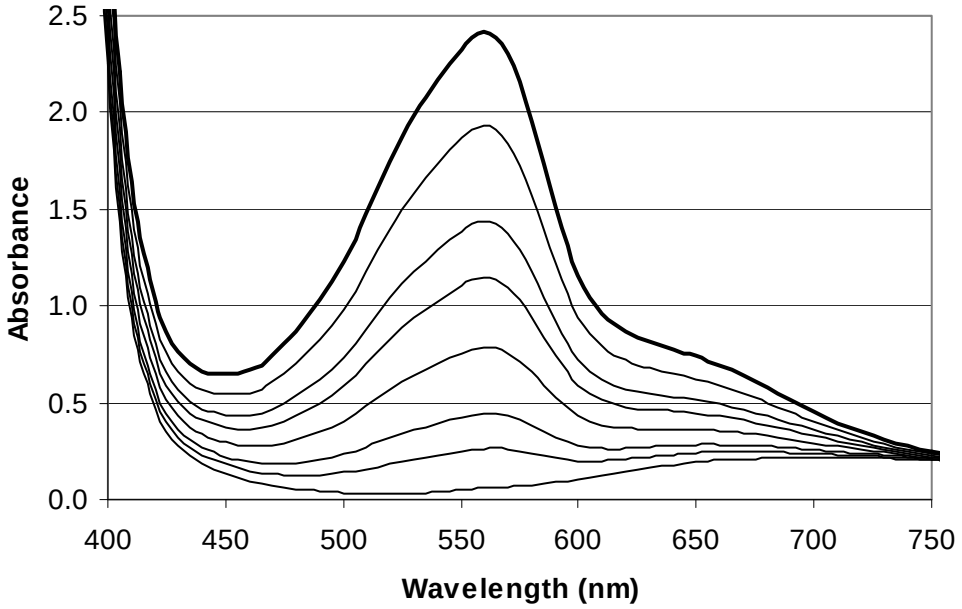

Figure 1. Absorbance spectra for BSA standards in the BCA Protein Assay (using standard test tube procedure). BSA standards are 0, 125, 250, 500, 750, 1000, 1500, 2000 µg/ml, respectively. The 2000 µg/ml line is drawn thicker than the others to orient the sequence.

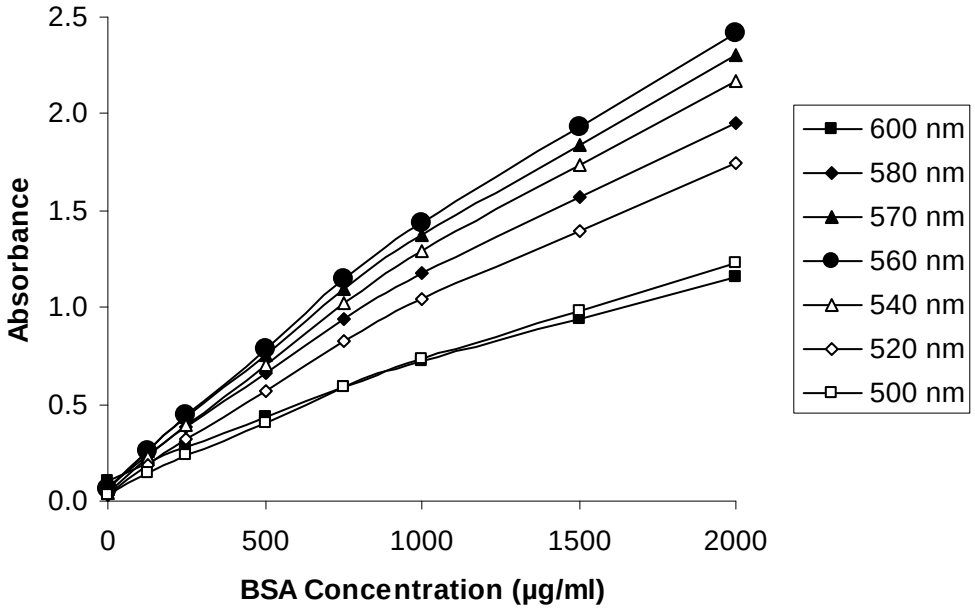

Figure 2. Standard curves for BSA standards in the BCA Protein Assay measured at selected wavelengths above and below the optimum (562 nm).

Coomassie Plus (Bradford) Protein Assay (Product No. 23236)

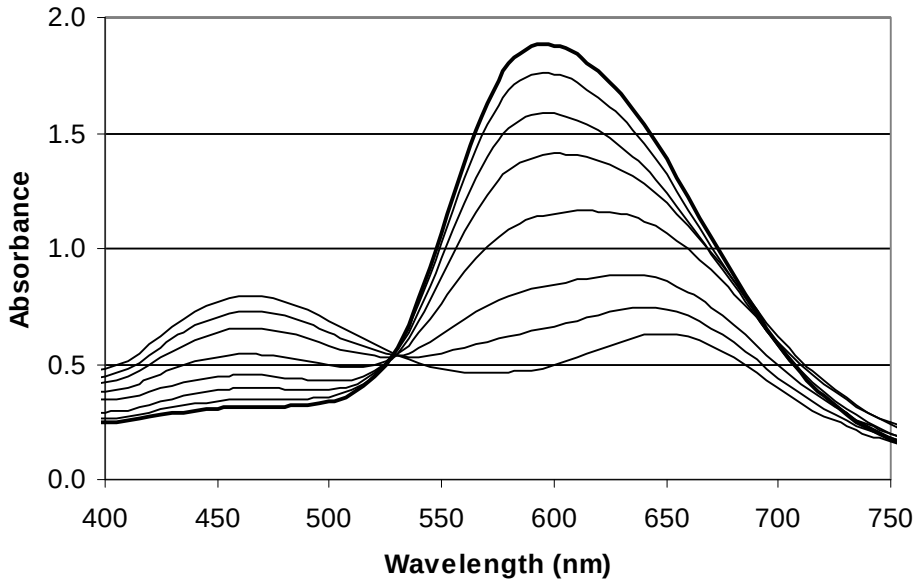

Figure 3. Absorbance spectra for BSA standards in the Coomassie Plus (Bradford) Assay (standard test tube procedure). BSA standards are 0, 125, 250, 500, 750, 1000, 1500, 2000 µg/ml, respectively. The 2000 µg/ml line is drawn thicker than the others to orient the sequence. Notice that an inverse relationship between protein concentration and absorbance occurs below 525 nm (maximum at 465 nm); concentration of immobilized protein can be estimated by measuring the decrease in dye absorbance at 465 nm (see related Tech Tip #9: Quantitate Immobilized Protein).

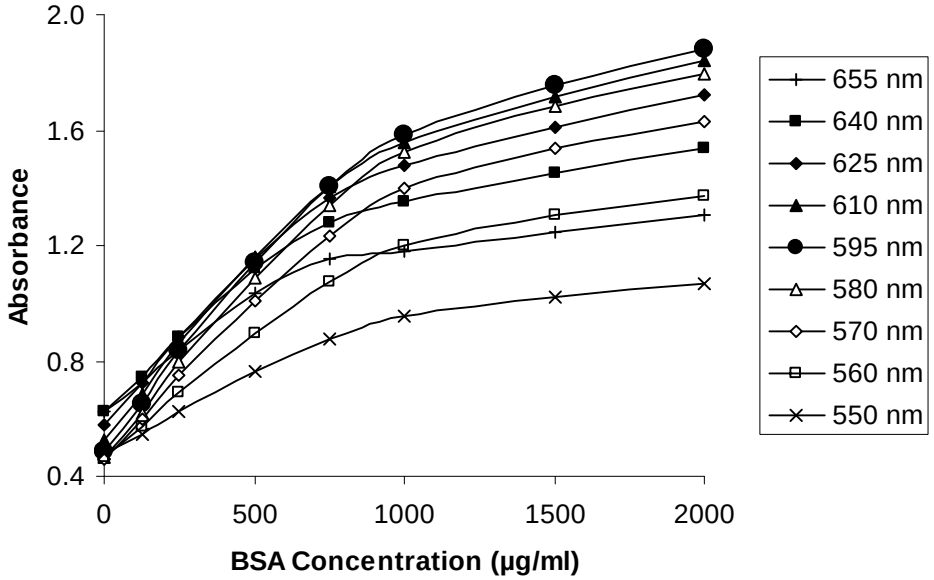

Figure 4. Standard curves for BSA standards in the Coomassie Plus (Bradford) Assay measured at selected wavelengths above and below the optimum (595 nm).

Pierce 660 nm Protein Assay (Product No. 22660, 22662)

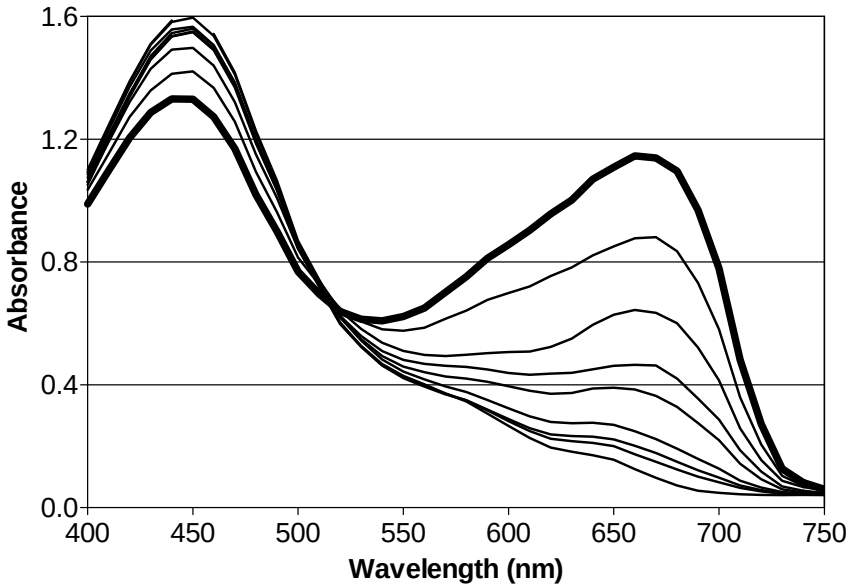

Figure 5. Absorbance spectra for BSA standards in the Pierce 660 nm Assay (standard test tube procedure). The BSA standards are 0, 50, 125, 250, 500, 750, 1000, 1500, 2000 µg/ml, respectively. The 2000 µg/ml line is drawn thicker than the others to orient the sequence.

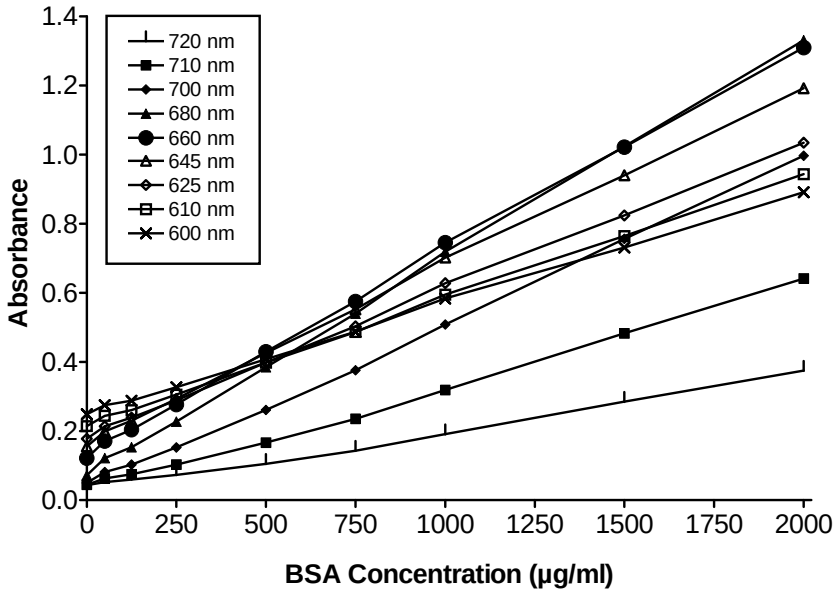

Figure 6. Standard curves for BSA standards in the Pierce 660 nm Assay measured at selected wavelengths above and below the optimum (660 nm).

Current versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.