

Title: Rapid Point-of Use Protein Assay: Kinetic analysis of BCA reaction using a portable reader

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Introduction

There has been a need for accurate estimation of sample protein concentration for many years. This was true during the development of early biochemical techniques, such as enzyme reactions, and later for assay of protein concentration in clinical samples, such as blood and urine. By necessity, rigorous methods were developed that employed laboratory spectrophotometers and multiplate readers. Simple absorbance at 280 nm was useful, although relatively insensitive, and is still used today for some applications. Several quantitative assays are now available that bring a high degree of precision and sensitivity. Assay reagents include dye-protein binding, such as Coomassie/Bradford reagents, or copper chelation, as in Lowry and Bicinchoninic Acid (BCA™). Each method has its advantages depending on the time required, possible interfering substances (buffers, salts, detergents or oxidizing agents) the concentration range, and protein-protein variation. Currently, each assay requires a daily preparation of a standard curve as well as negative controls, whether the analyst was testing one unknown sample or multiple samples. As the field of biotechnology continues to develop, protein assays play an ever increasing role in protein drug development, R&D, and clinical screening as well as process development, quality control and cleaning validation. The need for rapid information at each step in these areas has created the necessity for speed, which in turn has moved the location that protein assays have been performed closer to the point of use. For instance, in the research lab, rapid feedback on separation column fractions may influence what and how the next steps may be performed. On the production floor, assessment of key samples during fermentation could affect the production schedule as well as quality control. In this article, we will concentrate on the BCA assay and on a new method to kinetically read the assay, bringing protein assays closer to point-of-use.

Endosafe™-PTS BCA™ Technology

The Endosafe-PTS BCA test system utilizes a handheld reader and disposable test cartridge (figure 1) pre-loaded with precise amount of reagents from the Pierce BCA Protein Assay for the colorimetric detection and quantitation of total protein. The assay relies on the reduction of Cu^{+2} to Cu^{+1} by protein in an alkaline medium (the biuret reaction), followed by complexing of Cu^{+1} with two equivalents of bicinchoninic acid in which the early onset of color is detected and measured at an absorbance of 562nm by the PTS instrument.

A.



B.

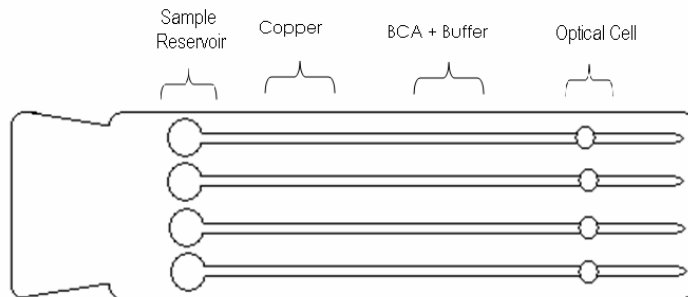


Figure 1 A. PTS (portable test system) instrument is shown with disposable cartridge (in bag) preloaded with all necessary reagents. B. Schematic diagram of disposable cartridge showing sample reservoir, reagent positions and optical cell area.

The PTS - BCA disposable test cartridge is shipped with the BCA reagents already dried on to the channels. Samples can then be added directly to the cartridge and the PTS instrument will measure the colorimetric change in each channel to quantify protein. Rather than reading the test as an end-point after a given incubation time, the PTS reads the kinetic development of color, measured by the decrease in transmission of light at 562 nm, the optimum absorbance maxima for BCA (figure 2). Each cartridge contains four channels containing concentrated cupric sulfate reagent and a concentrated alkaline reagent of bicinchoninic acid, sodium carbonate, sodium tartrate and sodium bicarbonate. Each cartridge is designed to test two samples in duplicate.

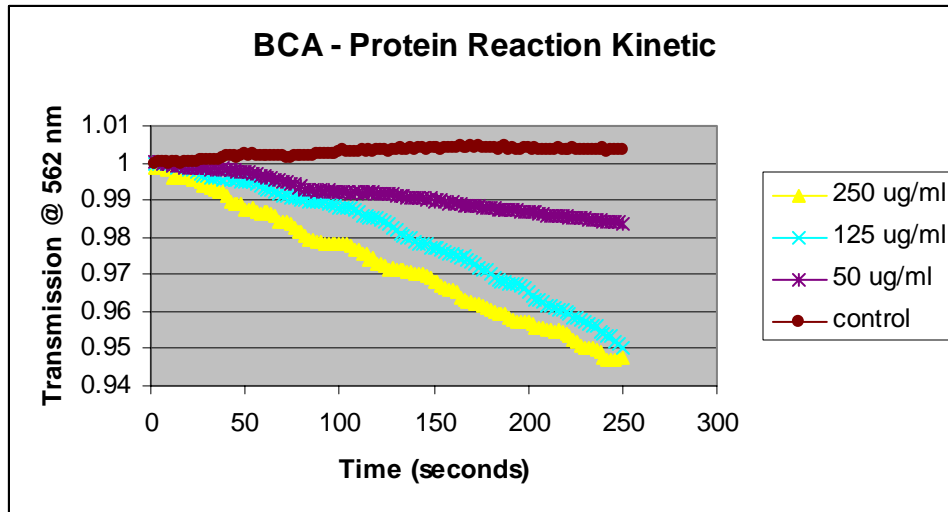


Figure 2 Reaction kinetics are shown for various concentrations of the protein Bovine Serum Albumin (BSA). The higher the protein concentration, the faster the reaction

Test Procedure

To perform the test, the user pipettes 25µL of a sample into two of the four sample reservoirs of the cartridge. There is no need to incubate protein sample prior to initiating the test. The PTS reader draws and mixes the sample with the BCA reagents. The sample is incubated and after mixing, the optical density (decrease in transmission) of the wells are measured and analyzed against an internally – archived standard curve (figure 3).

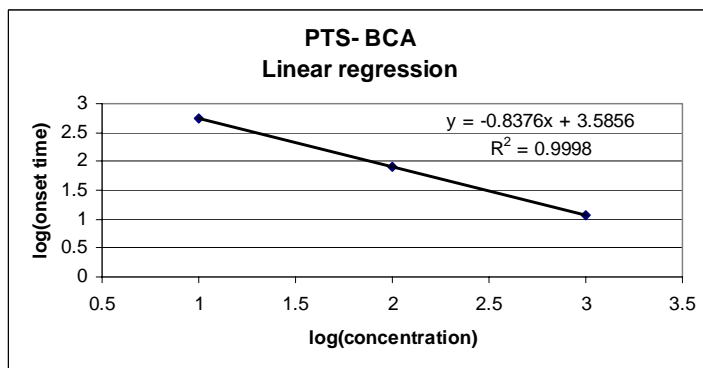


Figure 3. Example of an archived Bovine Serum Albumin standard curve with a range of 1000µg/mL to 10µg/mL. An archived standard curve is constructed using the log of the onset time vs. the log of the concentration for a given protein standard. The sample values are calculated by interpolation of the standard curve using the reaction times.

Elimination of Standard Curve Preparation

A primary advantage of the PTS-BCA is that comparable results of a conventional BCA protein assay are attainable without the need to prepare daily standard curves and working reagents. Technician variability is therefore minimized and assay time shortened. The PTS-BCA has two protein standards available: BSA (bovine serum

albumin) and BGG (bovine gamma globulin). The advantage of the archived standard curve is the reproducibility and accuracy of sample results over multiple days (table1).

Table 1. Example of PTS BCA Data Reproducibility

Sample	Day 1		Day 2		Day 3		Day 4		Day 5	
	Standard BCA	PTS BCA	Standard BCA	PTS BCA	Standard BCA	PTS BCA	Standard BCA	PTS BCA	Standard BCA	PTS BCA
Fetal Bovine Serum	28	37.4	35	34.4	32.8	37.4	35.1	35.8	27.7	37.4
		35.8		34.4		37.4		35.8		37.4
Fetal Bovine Serum	35	43.1	37	34.4	33.9	35.8	38.8	41.1	34.3	39.2
		41.1		34.4		34.3		39.2		33.0
Bovine Pituitary Extract	15.5	14.8	8.5	14.5	11.0	14.2	11.9	15.1	10.0	14.5
				12.7		15.8		14.2		15.5

Results in mg/mL

PTS - BCA run in duplicate

Data Analysis

With the PTS-BCA, data reporting is simple. At the conclusion of the test, the protein content for each sample is reported in $\mu\text{g/mL}$ and is displayed on the screen. The results can be downloaded to a computer and printed. Rather than the 80-110 minutes required for a standard Lowry, Coomassie or BCA end-point assay, the portable PTS BCA kinetic method delivers results in 15 minutes or less.

The Future

The technology behind the PTS was first developed for measuring bacterial endotoxins by Limulus Amebocyte Lysate (LAL). Kinetic analysis of that chromogenic enzymatic assay is enabling clinically important endotoxin testing in areas which were not possible in the past, with results that can be downloaded to computer workstations for logging and tracking data. The same principles can be applied to many biochemical and enzymatic assays currently encumbered by laboratory-bound equipment requiring computer analysis.