assays: protein

Bicinchoninic Acid (BCA) Method

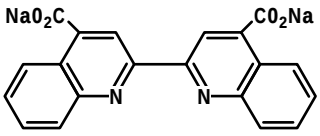
One of the most recent advances in protein assay technology is the use of bicinchoninic acid (BCA) in a unique reagent system. BCA is a highly sensitive and selective detection reagent for the cuprous cation (Cu^{+1}). This protein assay combines the well-known reduction of Cu^{+2} by protein to Cu^{+1} in an alkaline medium with the cuprous (Cu^{+1}) ion detecting property of BCA.[12]

The macromolecular structure of the protein, the number of peptide bonds and the presence of four amino acids (cysteine, cystine, tryptophan and tyrosine) have been reported to be responsible for color formation in protein samples when assayed with BCA. Studies with di-, tri- and tetrapeptides suggest that the extent of color formation is not the sum of the contributions of individual color-producing functional groups. Compounds with functional groups similar to those of cysteine, cystine, tyrosine or tryptophan are known to react with BCA.[13]

The purple-colored reaction product of this assay is formed by the interaction of two molecules of BCA with one cuprous ion (Cu^{+1}). This complex is water soluble and exhibits a strong absorbance at 562 nm, allowing the spectrophotometric quantitation of protein in aqueous solution.

The BCA method eliminates the need for precisely-timed reagent additions and vortexing inherent with the Lowry method, providing enhanced flexibility and ease of use. Additional advantages of the BCA method include: compatibility with ionic and nonionic detergents; a stable working reagent; less protein-to-protein variation; broad linear working ranges with excellent sensitivity; and the flexibility to easily change the protocol.

Figure 3: BCA M.W. 388.27



Reducing agents and copper chelators do interfere with this assay; however, interfering substances can be successfully eliminated prior to BCA analysis. This has been accomplished by precipitating the protein sample away from the interfering agents with TCA or cold acetone and redissolving the protein pellet in an SDS/NaOH solubilizing reagent or deionized water.[35-37] An alternative method describes the elimination of interfering substances such as thiols and reducing sugars prior to the BCA assay by binding proteins to positively charged nylon at alkaline pH and washing out the non-bound interfering agents.[38]

A report by Sorensen takes advantage of the interference of ascorbic acid with the BCA Protein Assay to ascertain the quality of a gel filtration desalting of a protein solution. The addition of ascorbic acid to the protein sample undergoing the desalting makes possible the identification of both the protein peak and the salt peak in one step.[39-40] Using the concept of BCA as a visual monitor of protein eluted from a gel filtration column, he has also developed a method to locate protein-enzyme conjugates. Because BCA does not interfere with enzyme-substrate reactions, tubes that give both a strong protein and strong enzyme signal indicate the presence of the conjugate.[40]

The BCA protein color reaction can be accelerated at room temperature with the addition of borate ions to the reagent.[14] This acceleration reduces the effect of some substances known to interfere in the standard and micro-BCA based assays. The faster BCA color reaction of this special formulation makes the assay adaptable to the kinetic mode and allows it to be run in automated or semi-automated systems.

The Pierce BCA Protein Assay Kit has been studied independently by Shibuya and coworkers,[41] demonstrating the dependence of the assay on different types of buffers and concentrations of alkali in both the standard and enhanced protocol. A BCA modification has been described that allows the rapid and reliable analysis of a large number of small volume (5 µl) samples using the commercial Pierce BCA kit in a microplate format with a microplate reader at dual wavelengths.[42] Others have taken the commerical kit and reported a modification that results in sensitive and reproducible protein determinations in seconds using a standard microwave oven.[43]

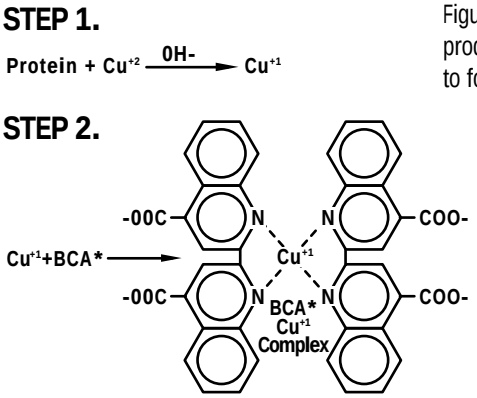


Figure 4: Proteins react with alkaline copper II to produce copper I. BCA then reacts with copper I to form an intense purple color at 562 nm.

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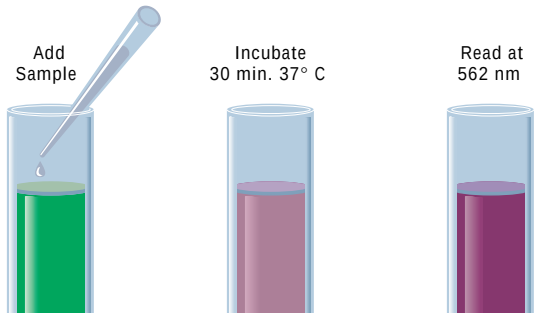
The Pierce patented BCA Protein Assay products deliver fast and reliable results with detergent-compatible chemistry. Three kits are available: the BCA Protein Assay Kit (Product #23225); the Micro-BCA Protein Assay Kit (Product #23235); and the Kinetic BCA Protein Assay Kit (Product #23250). Should you run low on one component included with your BCA Protein Assay Kit, replacement components for the BCA and Micro BCA Kits are also available The Pierce BCA Protein Assay products are unique and offer many advantages, including:

- Sample may contain detergents without interference
- Absolutely linear standard curve
- Easily modified sensitivity using time or temperature
- Low protein-to-protein variability

Each kit consists of two reagents that are mixed immediately before use and used within 24 hours. The kit components are stable for at least one year at room temperature. The apple green working reagent turns purple on reaction with proteins. Most assays are completed within 20-30 minutes. The longer the reaction is allowed to take, the better the sensitivity. Additional sensitivity can be achieved by incubating at elevated temperatures (up to 60°C has been used). Pierce has added borate to the buffers in its Kinetic BCA* Assay to offer further reduction of interference from substances often found in biological samples such as urine.

Figure 5: Standard Protocol for BCA Protein Assay Reagent.



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