assays: protein

Coomassie® Dye Binding Method

Another type of protein assay uses protein-binding dyes. The most popular is based on Coomassie® Brilliant Blue G-250 and is known as the Bradford Method.[10]

The Coomassie® Protein Assay is based on the immediate absorbance shift from 465 nm to 595 nm that occurs when Coomassie® Brilliant Blue G-250 binds to proteins in an acidic solution. Because the color response is non-linear over a wide range of protein concentrations, a standard curve must be run with each assay. Published reports discuss the possible causes for the non-linearity of the response and offer solutions . . . at the expense of sensitivity.[33,34]

The mechanism of the Coomassie® Dye Binding Assay has been examined.[31] The anionic form of the dye is the species that complexes with protein. Upon addition to a solution of protein, the dye binds to the protein, resulting in a color change from a reddish brown to blue. The dye has been assumed to bind to protein via electrostatic attraction of the dye's sulfonic acid groups.[1] The binding of protein to the anionic form of the Coomassie® dye results in an absorbance shift from 465-595 nm. The Coomassie® blue reagent has been shown to interact chiefly with arginine residues, but weakly with histidine, lysine, tyrosine, tryptophan and phenylalanine residues. VanderWaals forces and hydrophobic interactions also participate in the binding mechanism. The number of Coomassie® reagent ligands bound to each protein molecule is approximately proportional to the number of positive charges on the protein . . . about 1.5-3 dye molecules/charge.[11]

The assay is compatible with most agents that interfere with other assays, and it provides instant results. Detergents interfere with this protein assay,[28,29] but reducing agents and metals do not. The assay must be read quickly after its completion, because proteins will precipitate in the reagent over time, affecting the linearity of the response.

Although detergents are known to interfere with Coomassie® dye-based assays, the presence of low concentrations of non-ionic detergents (*i.e.* Triton® X-100) resulted in both improved sensitivity and protein-to-protein variability for the estimation of low molecular weight proteins.[30]

Another detergent anomaly has been reported for the glucopyranoside detergents. When used to solubilize membrane bound proteins, these detergents do not interfere with the Coomassie® protein assay.[32]

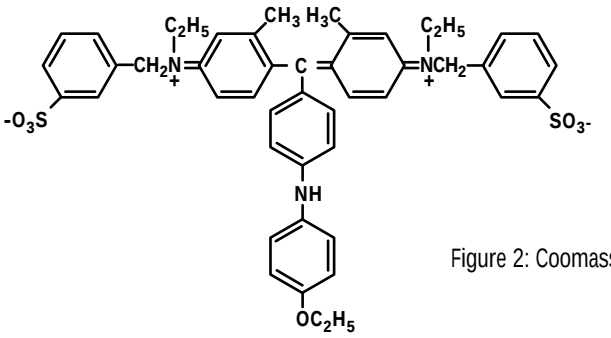

Figure 2: Coomassie Brilliant Blue G-250

assays: protein

Pierce has taken the Bradford Coomassie® Assay and made several improvements that offer unique advantages not available with other commercially-available Bradford assays. The result is available in The Pierce Coomassie® Protein Assay Reagent (Product #23200) and the Pierce Coomassie® Plus Protein Assay Reagent (Product #23236). Both Pierce products offer the following advantages:

- No preparation of reagent required . . . no dilution or filtering! The reagent is ready to use right out of the bottle!
- Extremely rapid assays. Results are possible in less than 30 seconds, and assays can be completed within minutes!
- Enhanced linear range with the Coomassie® Plus Protein Assay Reagent

The Pierce Coomassie® reagent allows the user to perform an actual protein assay in less than one minute. An assay that requires dilution and filtration would take 30 minutes!

References

1. Davies, E.M. (1988). Protein assays: A review of common techniques. *Am. Biotech. Lab.* 28-37.
10. Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254.
11. Tal, M., Silberstein, A. and Nusser, E. (1980). Why does Coomassie Brilliant Blue® interact differently with different proteins? *J. Biol. Chem.* **260**, 9976-9980.
28. Boccaccio, G.L. and Quesada-Allue, L.A. (1989). Interference of sodium dodecyl sulfate in the Bradford assay for protein quantitation. *An. Asoc. Quim. Argent.* **77(1)**, 79-88.
29. Carroll, K. and O'Kennedy, R. (1988). Interference effects from Nonidet P-40 and urea in the Bradford protein assay. *Biochem. Soc. Trans.* **16(3)**, 382-383.
30. Friedenauer, S. and Berlet, H.H. (1989). Sensitivity and variability of the Bradford protein assay in the presence of detergents. *Anal. Biochem.* **178(2)**, 263-268.
31. Compton, S.J. and Jones, C.G. (1985). Mechanism of dye response and interference in the Bradford protein assay. *Anal. Biochem.* **151(2)**, 369-374.
32. Fanger, B.O. (1987). Adaption of the Bradford protein assay to membrane-bound proteins by solubilizing in glucopyranoside detergents. *Anal. Biochem.* **162(1)**, 11-17.
33. Peterson, G.L. (1983). in *Methods in Enzymology.* Hirs, C.H.W. and Timasheff, S.N., eds. San Diego: Academic Press, Vol 91, pp 95-119.
34. Splittgerber, A.G. and Sohl, J. (1989). Nonlinearity in protein assays by the Coomassie blue dye-binding method. *Anal. Biochem.* **179(1)**, 198-201.