Technical Info

Well advised with Roth.

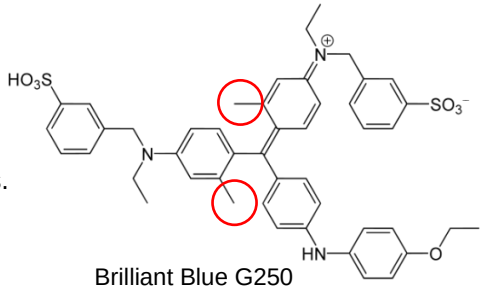
Coomassie™ Brilliant Blue - Mechanisms of Protein Staining and Bradford-Assay

Origin

The name Coomassie™ was first used in the late 19th century, adopted from the town of Coomassie™ (modern-day Kumasi in Ghana), as a trade name of the dye manufacturer Levinstein Ltd. for two similar triphenylmethane dyes used as acid wool dyes. The two blue dyes were then first produced in 1913 by Max Weiler based in Elberfeld, Germany. Today, the term 'Coomassie™' is a registered trademark of Imperial Chemical Industries.

Overall, there are approx. 40 dyes called 'Coomassie™ xy', while only Coomassie™ G250 and Coomassie™ R250 play a crucial role in biochemical analyses. During the last years, however, most authors referred to these dyes simply as 'Coomassie™' without specifying which dye is actually meant.

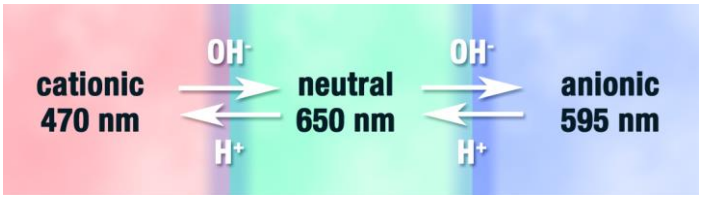
The term '250' originally was used for denotation of the purity of the dye. The suffix 'G' in 'Brilliant Blue G250' was added to describe the slightly greenish colour of the blue dye. The suffix 'R' in 'Brilliant Blue R250' is an abbreviation for 'red', as the blue colour of the dye has a slight reddish tint. Coomassie™ Brilliant Blue G250 differs from Coomassie™ Brilliant Blue R250 by the addition of two methyl groups.



Brilliant Blue G250

Background of Colour Changes

The colour of the two dyes depends on the acidity of the solution and on its binding status to amino acids or peptides. At a pH of less than 0 the dye has a **red** colour with an absorption maximum at a wavelength of 470 nm. At a pH of around 1 the dye is **green** with an absorption maximum at 650 nm while above pH 2 the dye is bright **blue** with a maximum at 595 nm.



The different colours result from the differently charged states of the dye molecule, corresponding to the amount of positive charges at the three nitrogen atoms present, while the two sulfonic acid groups are normally always negatively charged.

- At a pH of around zero, all three nitrogen atoms are positively charged, thus the dye will be a cation with an overall charge of +1, being in the **red** form.
- In the **green** form (pH of approx. 1) the dye will have no net overall charge (+2 and -2).
- At pH of 2 and more, up to the neutral pH, only one nitrogen atom carries a positive charge and the dye molecule is a **blue** anion with an overall charge of -1.
- Under alkaline conditions, the final proton is lost and the dye becomes **pink** in colour. This state, however, is of no relevance in biochemical assays.

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Mechanism of Gel Staining

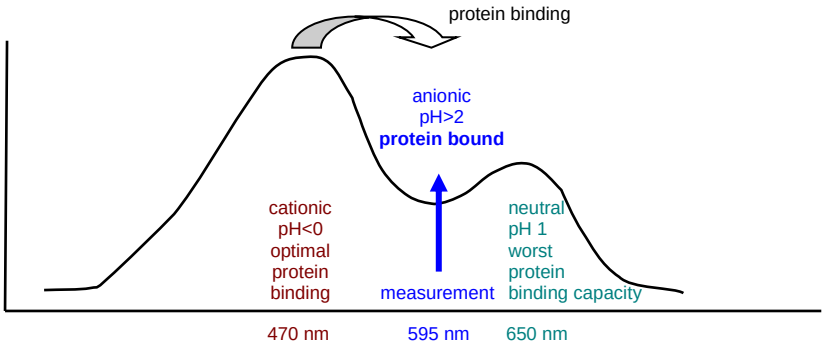
Visualisation of proteins by Coomassie™ Brilliant Blue R250 was first performed 1963 by Fazekas de St. Groth and colleagues (Fazekas de *et al.* (1963) *Biochim. Biophys. Acta* 71:377-91). Two years later, Meyer and Lambert used Coomassie™ Brilliant Blue R250 to stain proteins in a polyacrylamide gel (Meyer and Lambert (1965) *Biochim. Biophys. Acta 107:144-5).*

Coomassie™ Brilliant Blue forms strong but non-covalent complexes with proteins, most probably based on a combination of van der Waals forces and electrostatic interactions. Formation of the protein/dye complex stabilises the negatively charged anionic form of the dye producing the blue colour which may then be seen on the membrane or in the gel. The bound number of dye molecules is approx. proportional to the amount of protein present per band. However, binding of the Coomassie™ dyes to basic amino acids is much more efficient then to acidic amino acids; this effect may cause slight differences in staining of proteins in gels. When standard staining is used the gel matrix has to be destained subsequently, in order to visualize protein bands.

Modern gel staining solutions use a colloid form of the 'G' dye in solutions containing phosphoric acid (e.g. ROTI®Blue, Art. No. A152), in order to avoid the necessity to destain the gel (Diezel *et al.* (1972) *Anal. Biochem.* 48:617-20).

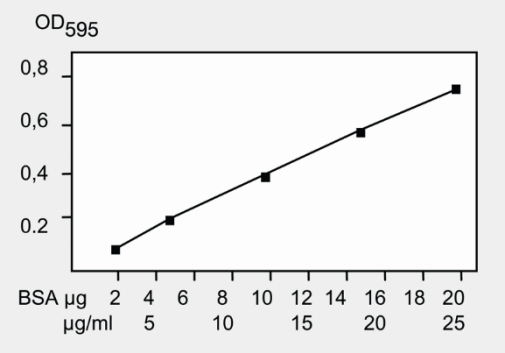
Mechanism of Bradford Assay

As mentioned above, the dye molecules bind to proteins to form a protein-dye complex. The Bradford assay uses the spectral properties of Coomassie Brilliant Blue G250 to estimate the amount of protein in a solution (Bradford M.M. (1976*) Anal. Biochem.* 72:248-54). In Bradford solutions, the dye is kept at a low pH in the red or greenish form; due to that mixture the Bradford solution itself looks brown.



Again, formation of the protein/dye complex stabilises the negatively charged anionic form of the dye producing the blue colour. The optical absorbance of the solution is measured at a wavelength of 595 nm. Thus, under standard conditions (proteins in PBS or other low concentrated salt solutions), highly linear standard curves may be produced.

This process, however, is altered by several putative factors. Binding of the Coomassie™ dyes to basic amino acids is much more efficient then to acidic amino acids. Thus, measurement of proteins with identical concentrations but significantly different amounts of basic amino acids may cause different data. Further, the binding to amino acids itself as well as the stabilization of the cationic, acidic form is hindered by several reagents that may be present in the assay like detergents, biological buffers, sugars etc. The dye also forms a complex with the anionic detergent sodium dodecylsulfate that stabilizes the neutral green form of the dye. These processes result in a signal shift during measurement that may well completely prohibit reliable analysis of the data.



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