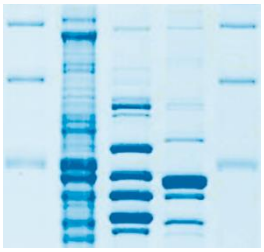
Technical INFO

CARL ROTH



Gel Staining of Protein Gels

Gel Staining Solutions

ROTH provides a large selection of high-quality staining kits and reagents for proteins, differing in their sensitivity and range of application. The table below will help you to select the staining method best suitable for your particular application.

Summaries: Staining of Proteins in Gels

Staining	Art. No.	Sensitivity	Description
Roti®-Black	L533	1 ng/mm²	**Silver staining kit.** Highly sensitive. Simple, fast and reproducible.
Roti®-Blue	A152	10 ng/mm²	**Coomassie-solution** for colloidal staining of proteins. **Destaining not required**. Non-fixing staining for protein gel elution possible. Proteomics-compatible.
Roti®-Blue quick	4829	10 ng/mm²	Solution for **rapid**, highly specific staining of proteins. **Destaining not required**. Non-fixing staining, compatible with blotting and Proteomics.
Roti®-White	K063	15 ng/mm²	**Zinc staining**. Fast and reversible. Proteins can be transferred onto a blotting membrane after staining. Protein gel elution possible.
Rotiphorese®-Blue R	3074	50 ng/mm²	**Coomassie** staining solution concentrate.

Staining	Sensitivity	Rapid Gel Analysis	Gel Drying	Elution / Transfer	2D-Gels	MS-Analysis
Roti®-Black P	1 ng/mm²	-	+++	-	+++	-
Roti®-Blue	10 ng/mm²	-	+++	+++	-	+++
Roti®-Blue quick	10 ng/mm²	+++	+++	++	-	++
Roti®-White	15 ng/mm²	+	-	+++	-	+
Roti®-Blue R	50 ng/mm²	+++	+	-	-	-

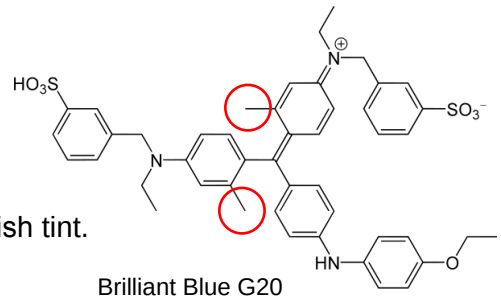
+++: recommended +: suitable -: not recommended

Coomassie™ Brilliant Blue

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.

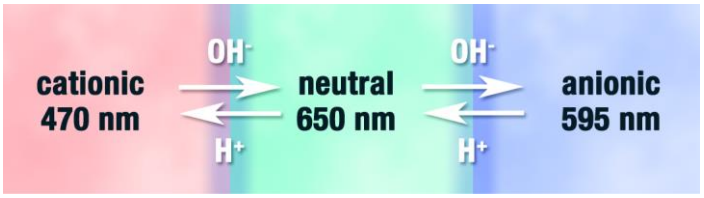
The term '250' originally was used for denotation of the purity of the dye. The suffix 'G' in 'Brilliant Blue G250' was added to describe the slightly greenish colour of the blue dye. The suffix 'R' in 'Brilliant Blue R250' is an abbreviation for 'red' as the blue colour of the dye has a slight reddish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



Brilliant Blue G20

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 620 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.

Mechanism of Gel Staining

Visualisation of proteins by Coomassie Brilliant Blue R-250 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 R-250 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).
Gel staining solutions Roti®-Blue, Rotiphorese®-Blue R and Roti®-Blue quick are based on this dye.

Carl Roth GmbH + Co. KG
Schoemperlenstraße 3-5
76185 Karlsruhe
Postfach 100121
76231 Karlsruhe
Telefon: +49 (0) 721/ 5606-0
Telefax: +49 (0) 721/ 5606-149
E-Mail: info@carlroth.de
Internet: www.carlroth.de
s.s. 04.2016