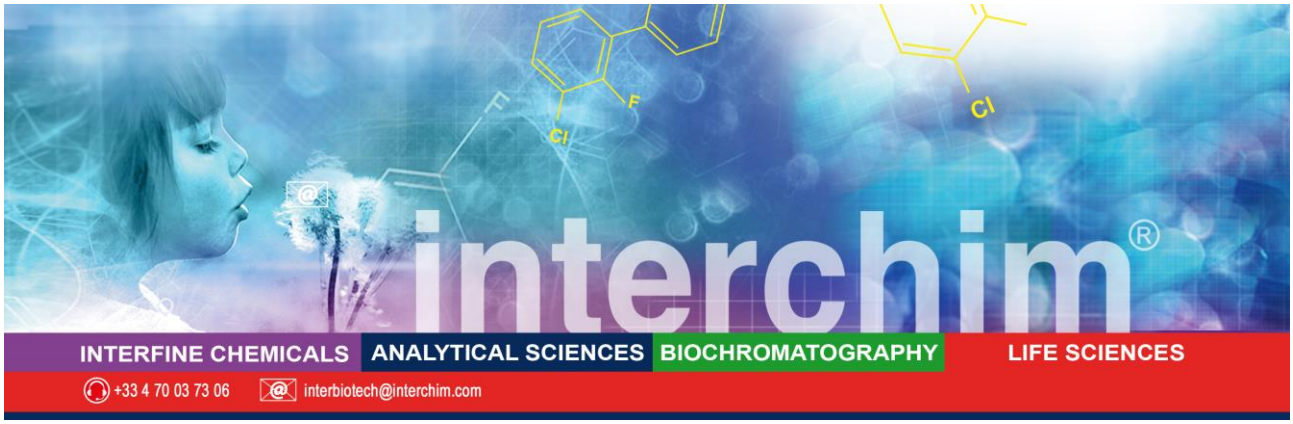
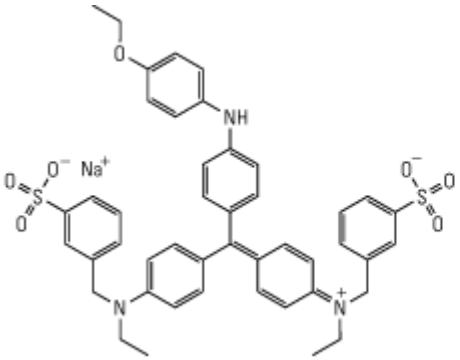
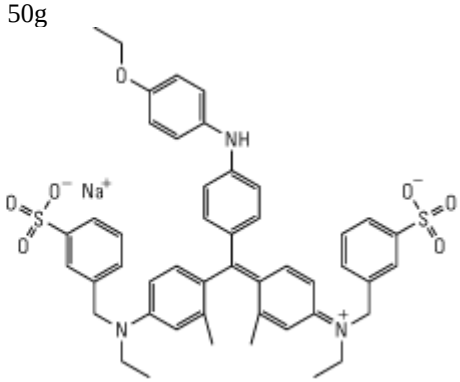
FT-115252



Coomassie Blue (R-250, G-250)

Products Description

	Coomassie Brillant Blue R-250	**Coomassie Blue G-250**
Name:		
Cat. Number	115252, 5g 115254, 25g	077582, 5g 077583, 25g 077584, 50g
		
Molecular formula :	$C_{45}H_{44}N_3NaO_7S_2$ (Sodium salt)	$C_{47}H_{48}N_3NaO_7S_2$ (Sodium salt)
CAS number:	6104-59-2	6104-58-1
MW:	825.97 g/mol	854,02 g/mol
Solubility:	essentially insoluble in water; dissolve first in methanol	slightly soluble in water; best to dissolve first in methanol or ethanol

Storage : Room temperature (2 years)

Applications: Protein staining in Gels
Protein assay in solution

Coomassie Blue is widely used in visualizing proteins separated by either agarose or acrylamide gel electrophoresis. The more popular is Coomassie R250 (Reddish tinted blue) for electrophoresis (more sensitive: can detect as little as 0.1 µg of protein), and Coomassie G250 (Greenish tinted blue) for protein assay in solutions (because it is more convenient - soluble). Now, both can be used in each application, however they can be used interchangeably because they stain proteins in somewhat different intensity and color.

Technical information:

Coomassie R-250 and G-250 dyes are two most common chemical forms of Coomassie dyes, as disulfonated triphenylmethane compounds. The R-250 (red-tinted) lacks two methyl groups that are present in the G-250 (green-tinted) form. The "250" originally denoted the purity of the dye.

The different colours are a result of the different charged states of the dye molecule.
At a pH of less than 0 the G250 dye caries all three nitrogen atoms with a positive charge, it has a red colour with an absorption maximum at a wavelength of 465 nm. At a pH of around 1 the dye bears an overall charge of +1 (the 2 sulfonic acid groups are negatively charge, thanks to theirextremely low pKa), so 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. At pH 7 the nitrogen atom of the diphenylamine moiety carries a positive charge, with an overall charge of −1, and the dye has an extinction coefficient of 43 000 M^{-1} cm^{-1}.[]
The dye interacts electrostatically with the amino and carboxyl groups of proteins (noncovalently), so from mainly basic aminoacids (arginine mostly, lysine, histidine). Hydrophobic interactions are also somewhat involved. (phenylalanine, tyrosine, tryptophane).

When dissolved in 0.01M citrate buffer at pH 3.0, Coomassie has an absorption maximum at 555nm; protein-dye complex is characterized by a peak slightly broader than that of the free dye with a maximum at 549nm. Hence, bound and unbound dye can be distinguished (principle of Bradford assay).

Typically Coomassie gel stains and protein Bradford-type assay reagents are formulated as very acidic solutions in 25 to 50% methanol. In acidic conditions, the dye binds to proteins primarily through basic amino acids (primarily arginine, lysine and histidine), while the formation of the complex stabilizes the negatively charged anionic form of the dye producing the blue colour. The number of Coomassie dye ligands bound to each protein molecule is approximately proportional to the number of positive charges found on the protein. Protein-binding causes the dye to change from reddish-brown to bright blue (absorption maximum equals 595nm).
The aqueous-solubility of the G-250 dye is taken to good account in protocols of colloidal Coomassie staining.

There is an interference from SDS detergent, especially with G250 dye (see alternative BC protein Assay for assaying proteins with SDS).

Directions for Use

Protein assay in solution – Bradford

● Stain solution composition:
5% Coomassie Blue G250

Stain solution preparation:
1. Dissolve 50mg of Coomassie Blue G250 in 50ml of methanol
2. Add 100ml of 85% H_3PO_4 to the solution from step 1
3. Add the solution from step 2 into 500ml of H2O and mix well
4. Filter to remove any precipitates
5. Add an additional 350ml of H_2O and miw
   Store at 4°C.

● Procedure for a standard Assay, 20-150 µg protein; 200-1500 µg/mL

Prepare a series of protein standards diluted with 0.15 M NaCl to final concentrations of 0 (blank = NaCl only), 250, 500, 750 and 1500 µg/mL. Also prepare serial dilutions of the unknown sample to be measured.
Add 100 µL of each of the above to a separate test tube (or spectrophotometer tube).
Add 5.0 mL of Coomassie Blue to each tube and mix by vortex, or inversion.
Adjust the spectrophotometer to a wavelength of 595 nm, and blank using the tube which contains no protein.
Wait 5 minutes and read each of the standards and each of the samples at 595 nm wavelength.
Plot the absorbance of the standards vs. their concentration. Compute the extinction coefficient and calculate the concentrations of the unknown samples.

For more convenience and optimized sensitivity and linearity, see **CooAssay reagent** UPF86400.

Coomassie protein gel stain

● Stain solution composition:
45% Methanol (reagent grade)
10% Glacial acetic acid
45% Water
3g/L Coomassie Brilliant Blue R250

Stain solution preparation:
Add 100mL of glacial acetic acid to 450mL ultrapure water.
Dissolve the 3g of Coomassie Dye in 450mL methanol.
Filter the solution before use

● Standard Gel staining Protocol

1- Gel may be prefixed in 50% MeOH, 10% HoAC, 40% H_2O for 30 minutes to overnight.

2- Stain gel in the above solution, with 0.25-0.3% Coomassie Blue R-250, for 2 - 4 hours, until the gel is a uniform blue color. Staining is complete when the gel is no longer visible in the dye solution. Prior to complete staining, the gel will appear as a lighter area against the dark staining solution.

3- Destain for 4 - 24 hours in 5% MeOH, 7.5% HOAC, 87.5% H_2O. Bands will begin to appear in 1 - 2 hours. Destain until background is clear. Note: This method will detect as little as 0.1µg/band.

4- Store gels in 7% HOAC.

● Rapid Protocol

1- Fix gel in 25% IPA, 10% HOAC in water for 30 - 60 minutes.

2- Stain gel in 10% acetic acid in water, containing 60 mg/L of Coomassie Blue R-250. Bands will appear in 30 minutes. Allow staining to proceed until desired band intensity is reached. In this protocol, background staining is low due to the very low dye concentration used.

3- Destain gel in 10% acetic acid for 2 hours or more. Store gels in 7% HOAC.

Blue Native PAGE

Taking to good account the negative charge of Coomassie dye bound to proteins, the Coomassie can be used to separate protein complexes using polyacrylamide gel electrophoresis under non-denaturing conditions in a technique called Blue Native PAGE.

For more convenience and optimized sensitivity and linearity, see **CooAssay Protein Gel stain** UPF86400:
Destain Solution (for Coomassie stained gels) 1J3460, 1L
Removes the excess gel stain within Poly-acrylamide Gels (PAGE), leaving blue protein stained bands within a clear gel.

Related products :

- 3Dye 2D DIGE Kit, EV0870

Legals :

Coomassie is a registered trademark of Imperial Chemical Industries.

Ordering information

Further package sizes and pricing may be found at http://www.interchim.com
Please inquire for bulk quantities (availability, shipment conditions, etc) at uptima@interchim.com

Any questions please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06

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interchim 211 bis, avenue JF Kennedy BP 1140 - 03100 Montluçon Fax +33 4 70 03 82 60 Hotline +33 4 70 03 73 06 • interbiotech@interchim.com

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