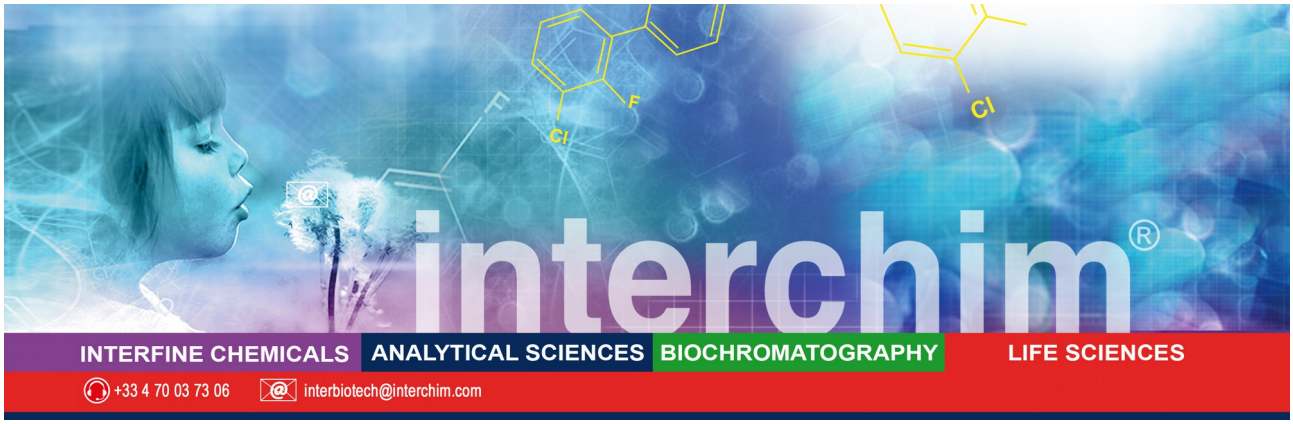
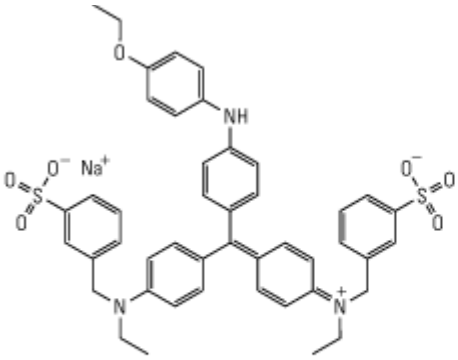
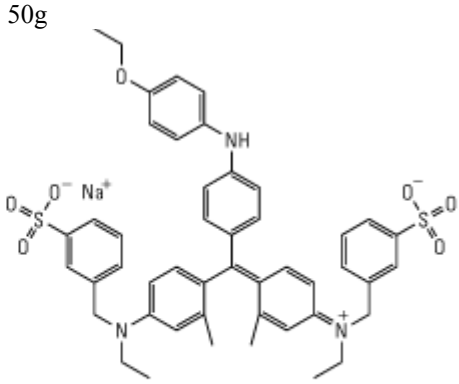


Coomassie Blue (R-250, G-250) Protein Gel Stains

Products Description

Name:	**Coomassie Brillant Blue R-250**	**Coomassie Blue G-250**
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)

Coomassie Blue is widely used in visualizing proteins separated by either agarose or acrylamide gel electrophoresis. The more popular Coomassie R-250 can detect as little as 0.1 µg of protein. It is more sensitive than G-250 which is more convenient (solubility).

Technical information:

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

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).
There is an interference from SDS detergent, especially with G250 dye (see alternative BC protein Assay).
The aqueous-solubility of the G-250 dye is taken to good account in protocols of colloidal Coomassie staining.

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).

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Directions for Use

Recipe for simple coomassie protein gel stain.

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

Stains 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.

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Protein assay in solution – Bradford

Stains solution composition:
5% Coomassie Blue G250

Stains solution preparation:
Dissolve 50mg of Coomassie Blue G250 in 50ml of methanol
Add 100ml of 85% H_3PO_4 to the solution from step 1
Add the solution from step 2 into 500ml of H2O and mix
Filter to remove and precipitates
Add an additional 350ml of H_2O
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.

Related products :

CooAssay Protein Gel stains, G4562A, 500ml
colloidal formulation, rapid stain and non destain or water destain)
Destain Solution (for Coomassie stained gels - glacial acetic acid and methanol base) 1J3460, 1L
Removes the excess gel stain within Poly-acrylamide Gels (PAGE), leaving blue protein stained bands within a clear gel.

- Protein Low Range (3,5-31 kDa, 6 bands), 587231
- BLUEYE PRESTAINED PROTEIN LADDER, FO9810
- GD PRESTAINED PROTEIN LADDER, XZ2310
- PINK PLUS PRESTAINED PROTEIN LADDER, XZ2300
- PINK PRESTAINED PROTEIN LADDER, XZ2290
- 3Dye 2D DIGE Kit, EV0870

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).

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

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