

Chromeo™ P503

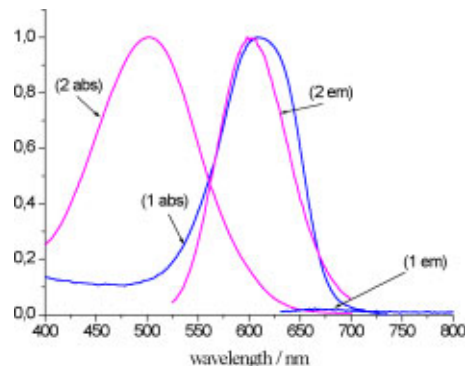
Catalog No: 15106, 16106

Chemical Properties:

CONTENTS: Supplied as a 1 mg (Cat # 15106) or 5 mg (Cat # 16106) lyophilized blue solid.

Soluble in DMF, methanol and acetonitrile.

MW: 393.29; m.p. >300 °C;



The blue curves (1) represent the absorption and emission spectra of free label, whereas the red curves (2) are characteristic for conjugated Chromeo P503.

Fluorescent Properties:

Chromeo P503 detects proteins and peptides by exhibiting a color change from blue to red upon binding to primary amines. On conjugation to the primary amino groups, the label undergoes a shortwave spectral shift of more than 100 nm. Chromeo P503 displays a weak fluorescence with a quantum yield below 1 % in solution. On conjugation to the amine, the quantum yield rises to 50 %. This property allows a distinct detection of primary amines, proteins and other bio-molecules.

Absorption: 612 nm (free), 503 nm (conjugated)

Emission: 665 nm (free), 600 nm (conjugated)

ϵ L/(mol·cm): 60 000 (free), 35 000 (conjugated)

Quantum Yield: < 1 % (free), ~ 50 % (conjugated)

Quality Control:

The Dye has been quality tested by conjugation to BSA and spectro-photometrical evaluation.

Storage and Guarantee:

To ensure stability, the lyophilized dye should be stored at 4°C in the dark. This product is guaranteed for 6 months from the date of arrival.

Protocol for labeling proteins with Chromeo P503

Preparation of the working solution

Dissolve 1 mg of Chromeo P503 in 100 μ l of dimethylformamide (DMF). Do not use amine-containing solutions or buffers as a solvent. The stock solution can be stored in the dark at 4°C for 6 months .

Labeling reaction

Dissolve 2 mg of HSA (or another protein) in 0.5 ml of bicarbonate buffer (0.1 M, preferably of pH 9.0) and add 5-10 μ l of the working solution drop-wise to the protein solution. Gently stir the reaction mixture at room temperature for 30 minutes.

The reactive dye in solution is blue. The blue colour disappears and becomes yellow, when the dye is stored in a basic solution.

Bicarbonate buffer, pH 9.0

2.1 g of NaHCO₃ are dissolved in 500 ml doubly distilled water. The buffer is adjusted to pH 9.0 with 1 N NaOH.

Purification of the conjugated protein

For some applications the purification of the dye conjugated protein may be necessary.

The labeled protein is purified by size-exclusion chromatography using Sephadex G25 as stationary phase and phosphate buffer of pH 7.2 (22 mM) as the eluent. The red band indicates the labeled protein.

Phosphate buffer (22mM), pH 7.2

5.67 g Na₂HPO₄ x 12 H₂O and 0.96 g NaH₂PO₄ x 2H₂O are dissolved in 1 L of ddH₂O. The buffer is adjusted with 1 N HCl to pH 7.2.