Chromeo™ P543

Catalog No: 15109, 16109

Format: 1 mg, 5 x 1 mg

Chemical Properties: Contents: Supplied as a 1 mg (Cat. No. 15109) or 5 x 1 mg (Cat. No. 16109) lyophilized violet solid. Soluble in DMF, methanol and acetonitrile.

Net formula: $C_{18}H_{18}NOS^{+}\ BF_4^{-}$; MW 383,41; melting point: 259°C

Fluorescent Properties: Chromeo P543 detects proteins and peptides by exhibiting a color change from violet to dark red upon binding to primary amines. On conjugation to the primary amino groups, the label undergoes a shortwave spectral shift of 27 nm. Chromeo P543 displays a weak fluorescence with a quantum yield below 0.5% in solution. On conjugation to the amine, the quantum yield rises to 15%. This property allows a distinct detection of primary amines, proteins and other bio-molecules.

Absorption: 570 nm (free), 543 nm (conjugated)

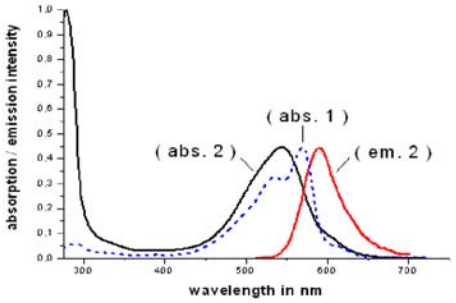
Emission: Non-detectable (free), 590 nm (conjugated)

e L/(mol·cm): 110,000 (free), 57,000 (conjugated)

Quantum Yield: 0% (free), ~15% (conjugated, depending on the DPR of the protein conjugate)

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.



The blue line (abs. 1) represents the absorption spectrum of the free label, the black line (abs. 2) the conjugated form. The red line (em. 2) shows the fluorescent emission spectrum of the conjugated Chromeo P543.

Protocol: Protocol for labeling proteins with Chromeo P543

Preparation of the working solution
Dissolve 1 mg of Chromeo P543 in 100 µl 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 8.3) and add 5 µl of the working solution drop-wise to the protein solution. Gently stir the reaction mixture at room temperature for 1 hour.
The reactive dye in solution is violet. The violet color disappears and becomes yellow when the dye is stored in a basic solution.

Bicarbonate buffer of pH 8.3
4.2 g of $NaHCO_3$ are dissolved in 500 ml doubly distilled water. The buffer is adjusted to pH 8.3 with 1 N NaOH. (The dye shows high reactivity in a pH range from 8.0 to 9.0)

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, pH 7.2 (22 mM) as the eluent. The red band indicates the labeled protein.

Phosphate buffer (22 mM), pH 7.2
5.67 g Na_2HPO_4 x 12 H_2O and 0.96 g NaH_2PO_4 x $2H_2O$ are dissolved in 1 L of ddH_2O. The buffer is adjusted with 1 N HCl to pH 7.2.