

## Chromeo™ P540

**Catalog No:** 15107, 16107

**Format:** 1 mg, 5 x 1 mg

**Chemical Properties: Contents:** Supplied as a 1 mg (Cat. No. 15107) or 5 x 1 mg (Cat. No. 16107) lyophilized blue solid. Soluble in DMF, methanol and acetonitrile.

Net formula:  $C_{25}H_{26}NO^+ BF_4^-$ ; MW: 433.29; melting point: 290°C

**Fluorescent Properties:** Chromeo P540 detects proteins and peptides by exhibiting a color change from purple to red upon binding to primary amines. On conjugation to the primary amino groups, the label undergoes a shortwave spectral shift of 54 nm. Chromeo P540 displays a weak fluorescence with a quantum yield below 1% in solution. On conjugation to the amine, the quantum yield rises to 20%. This property allows a distinct detection of primary amines, proteins and other bio-molecules.

**Absorption:** 587 nm (free), 533 nm (conjugated)

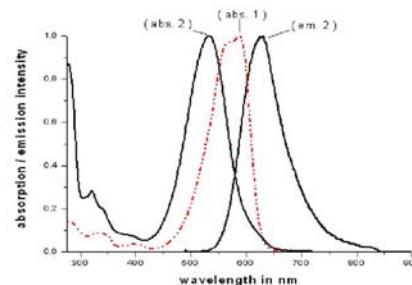
**Emission:** Non-detectable (free), 627 nm (conjugated)

**$\epsilon$  L/(mol·cm):** 80,000 (free) in aqueous solution, 50,000 (conjugated) in aqueous solution

**Quantum Yield:** 0% (free), ~20% (conjugated, depending on the DPR of the conjugated protein)

**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 red line (abs. 1) is the absorption spectrum of free label. The black lines are the absorption spectrum (abs. 2) and the fluorescent emission spectrum (em. 2) of conjugated Chromeo P540.

**Protocol: Protocol for labeling proteins with Chromeo P540**

**Preparation of the working solution**

Dissolve 1 mg of Chromeo P540 in 100  $\mu$ l of dimethylformamide (DMF). Do not use amine-containing solutions or buffers as 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 5-10  $\mu$ l 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 purple. The purple color disappears and becomes yellow, when the dye is stored in basic solution.

**Bicarbonate buffer of pH 8.3**

4.2 g of  $\text{NaHCO}_3$  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  $\text{Na}_2\text{HPO}_4 \times 12 \text{H}_2\text{O}$  and 0.96 g  $\text{NaH}_2\text{PO}_4 \times 2\text{H}_2\text{O}$  are dissolved in 1 L of ddH<sub>2</sub>O. The buffer is adjusted with 1 N HCl to pH 7.2.