

Chromeo™ P465

Catalog No: 15105, 16105

Format: 1 mg, 5 x 1 mg

Chemical Properties: Contents: Supplied as a 1 mg (Cat. No. 15105) or 5 x 1 mg (Cat. No. 16105) lyophilized blue solid. Soluble in DMF, methanol and chloroform.

Net formula: $C_{19}H_{22}NO^+ BF_4^-$; MW 367.19

Fluorescent Properties: Chromeo P465 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 P465 displays a weak fluorescence with a quantum yield below 1% in solution. On conjugation to the amine, the quantum yield rises to 14%. This property allows a distinct detection of primary amines, proteins and other bio-molecules.

Absorption: 645 nm (free), 465 nm (conjugated)

Emission: 732 nm (free), 630 nm (conjugated)

ϵ L/(mol·cm): 25,000 (free), 25,000 (conjugated)

Quantum Yield: less than 1% (free), ~14% (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.

Protocol: Protocol for labeling proteins with Chromeo P465**Preparation of the working solution**

Dissolve 1 mg of Chromeo P465 in 100 µl of dimethylformamide (DMF). The dye is very sensitive to amines : Do not use amine-containing solutions or buffers as solvents. 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 10-20 µl 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 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 $\text{Na}_2\text{HPO}_4 \times 12 \text{H}_2\text{O}$ and 0.96 g $\text{NaH}_2\text{PO}_4 \times 2\text{H}_2\text{O}$ are dissolved in 1 L of ddH₂O. The buffer is adjusted with 1 N HCl to pH 7.2.