

Chromeo™ P429

Catalog No: 15108, 16108

Format: 1 mg, 5 x 1 mg

Chemical Properties: Contents: Supplied as a 1 mg (Cat. No. 15108) or 5 x 1 mg (Cat. No. 16108) lyophilized yellow/brown solid. Soluble in DMSO. Chromeo P429 is soluble in other solvents such as DMF, methanol and chloroform, but at lower concentrations.

Net formula: $C_{16}H_{16}NOS^+ ClO_4^-$; MW 369.82; melting point: 190°C

Fluorescent Properties: Chromeo P429 detects proteins and peptides by exhibiting a color change upon binding to primary amines. On conjugation to the primary amino groups, the label undergoes a shortwave spectral shift of 28 nm. Chromeo P429 displays a weak fluorescence with a quantum yield below 0.5% in solution. On conjugation to the amine, the quantum yield rises to 10%. This property allows a distinct detection of primary amines, proteins and other bio-molecules.

Absorption: 457 nm (free), 429 nm (conjugated)

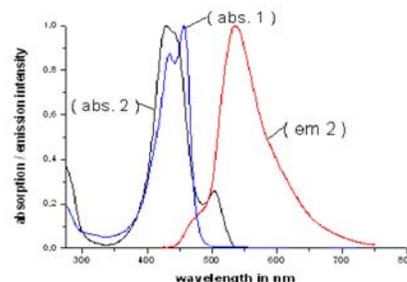
Emission: Non-detectable (free), 536 nm (conjugated)

ϵ L/(mol·cm): 65,000 (free) in aqueous solution, 75,000 (conjugated) in aqueous solution

Quantum Yield: 0% (free), ~10% (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 blue line (abs. 1) is the absorption spectrum of free label, the black line (abs. 2) of the conjugated form. The red line (em. 2) shows the fluorescent emission spectrum of conjugated Chromeo P429.

Protocol: Protocol for labeling proteins with Chromeo P429**Preparation of the working solution**

Dissolve 1 mg of Chromeo P429 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 1-2 hours.

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 ddH_2O . The buffer is adjusted with 1 N HCl to pH 7.2.