

Chromeo™ 488 NHS-Ester

Catalog No: 15511, 16511

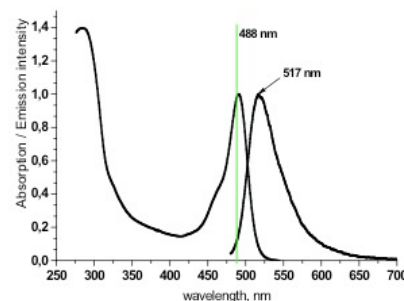
Chemical Properties:

Contents: 1 mg (Catalog Number 15511) or 5 mg (Catalog Number 16511) of lyophilized Chromeo™ 488 NHS Ester

Net formula: $C_{26}H_{26}ClO_9$; MW 521.9

Reagent color: yellow-orange

Soluble in DMF, DMSO, ethanol and methanol.



Spectrum of Chromeo™ 488 NHS-Ester conjugated to BSA in PBS.

Fluorescent Properties:

Chromeo 488 can be excited with a green laser and shows similar fluorescent properties as fluorescein or other 488-excitable dyes. Filters designed for fluorescein can be used with Chromeo 488 and its conjugates. Chromeo 488 exhibits a large tolerance to pH and an unmatched photostability. This allows more time for image capture.

Molar extinction Coefficient: 73,000 $M^{-1}cm^{-1}$ (measured at A_{max})

Quantum Yield when conjugated to BSA: 38%

Excitation Wavelength: 488 nm

Emission Wavelength: 517 nm

Important product information

NHS-activate dyes are very sensitive to moisture. They should be stored in the original vial at $-20^{\circ}C$ in the original packaging. To avoid a decrease in activity by moisture condensation, the vial should be slowly brought to room temperature before opening.

Prepare the dye-stock solution immediately before use. Do not store the stock-solution containing the activated dye.

Quality Control:

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

Storage and Guarantee:

To ensure stability, the lyophilized dye should be stored at $-20^{\circ}C$ in the dark. As the dye is moisture sensitive, it should be stored in the original foil pouch with dessicant. This product is guaranteed for 6 months from the date of arrival.

Protocol for labeling proteins with Chromeo 488 NHS-Ester

The procedure listed below represents a guideline for the use of reactive Chromeo-Dyes. The total number and the surface exposition of free amines will vary from protein to protein. Therefore we recommend to optimize the labeling reaction for each target by using different molar ratios of protein to reactive-dye. In general 1 mg of Chromeo 488 NHS-Ester allows labeling of 15 mg of protein.

Preparation of the Dye-Working Solution

Dissolve 1 mg of Chromeo 488-NHS in 10 μ l of DMF.

NOTE:

1. The dye-containing tube should reach room temperature before opening.
2. The stock solution is not suitable for storage. Please use up within 1 hour. Optimal yields of conjugated proteins can be guaranteed, if the dye is dissolved immediately before starting the labeling procedure.

Preparation of the protein solution

Dissolve the appropriate amount of protein in 1 ml of 50 mM Bicarbonate buffer, preferable of pH 8.3. Usually the protein concentration should be 2-20 mg/ml.

NOTE: Buffers containing primary amines (e.g. Tris or glycine) are not compatible, they will react with the NHS-esters and decrease the amount of conjugated protein.

Bicarbonate buffer, pH 8.3

2.1 g of NaHCO₃ are dissolved in 500 ml doubly distilled water. The buffer is adjusted to pH 8.3 with 1 N NaOH or HCl.

Labeling reaction

1. While gently stirring the protein solution, add 1-10 μ l of the Dye-Working Solution drop-wise into it. (Mix the solution carefully with the pipette-tip and release the dye slowly into it).
2. Incubate 1 hour at room temperature.

If 5 mg of protein are dissolved in 1 ml of Bicarbonate buffer, approximately 10 μ l of Dye-Working Solution should be added. Due to protein-protein variations of reactivity, we recommend to optimize by testing different dye-to-protein ratios.

Purification of the conjugated protein

Purify the labeled protein by size-exclusion chromatography using Sephadex G25 as stationary phase and PBS or any buffer of choice, which should not contain free amino groups as eluent. The first colored band (the excluded fraction of the chromatography) will be the labeled protein.

Degree of Labeling

The dye-to-protein ratio (DPR) indicates the number of dye molecules attached per protein molecule. It is calculated by the means of the absorption at 280 nm, the absorption of the dye at its maximum and the molar coefficients. The dye-to-protein ratio contributes to the brightness of a conjugate, although it can be influenced and reduced by quenching when dye molecules are localized too close to each other. Optimization of the DPR might be needed to increase the brightness of your conjugate. In our hands a DPR of 3.0 is optimal for conjugates with Chromeo 488.

To calculate the DPR, measure the absorption of the conjugate at 280 nm (A_{280}) and the absorption of the dye at its maximum at 488 nm ($A_{488-max}$) by using a 1 cm path length cuvette.

$$DPR = C_{488} / C_{protein}$$

C: molar concentration

$$C_{494} = A_{488-max} / \epsilon_{488-max}$$

$\epsilon_{494-max}$: molar extinction coefficient Chromeo 488 at 494 nm = 73,000 M⁻¹cm⁻¹

$$C_{protein} = A_{protein} / \epsilon_{protein}$$

$\epsilon_{protein}$: molar extinction coefficient of the protein: 200,000 M⁻¹cm⁻¹ for antibodies; 43,800 M⁻¹cm⁻¹ for BSA; 37,000 M⁻¹cm⁻¹ for lysozyme

$$A_{protein} = A_{280} - (A_{488-max} \times \epsilon_{488-280} / \epsilon_{488-max})$$

$\epsilon_{488-280}$: molar extinction coefficient of Chromeo 488 at 280 nm = 12,000 M⁻¹cm⁻¹