

Chromeo™ 546 NHS-Ester

Catalog No: 15211, 16211

Format: 1 mg, 5 x 1 mg

Chemical Properties: Contents: 1 mg (Catalog No. 15211) or 5 x 1 mg (Catalog No. 16211) of lyophilized Chromeo™ 546 NHS-Ester.

Net formula: $C_{39}H_{50}N_3O_7P$; MW 703.82

Reagent color: purple solid.

Soluble in DMF, ethanol, methanol and acetonitrile.

Fluorescent Properties: Chromeo 546 shows similar fluorescent properties as the yellow-orange Cy3 dye. Filters designed for Cy3 can be used with Chromeo 546 and its conjugates. Chromeo 546 exhibits a large tolerance to pH and unmatched photostability, which provides more time for image capture.

Molar Extinction Coefficient: $96,800 \text{ M}^{-1} \text{ cm}^{-1}$ (measured at A_{max})

Quantum Yield when conjugated to BSA: 37%

Excitation Wavelength: 545 nm

Emission Wavelength: 561 nm

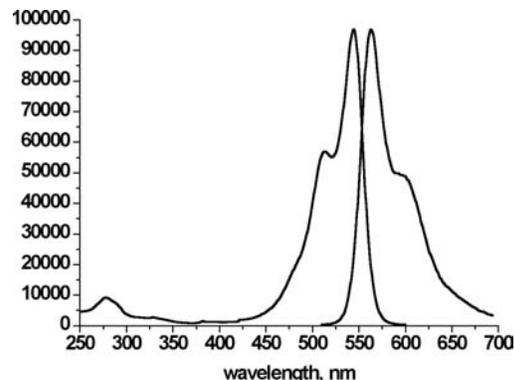
Important product information:

NHS-activated dyes are very sensitive to moisture. They should be stored in the original pouch at -20°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.

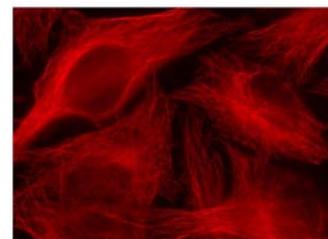
Prepare the dye-stock solution immediately before use. Do not store the stock-solution containing the activated dye.

Quality Control: The Dye has been quality tested by TLC, by spectrophotometrical evaluation and test-conjugation to BSA.

Storage and Guarantee: To ensure stability, the lyophilized dye should be stored at -20°C in the dark. As the dye is moisture-sensitive, it should be stored in the original foil pouch with desiccant. This product is guaranteed for 6 months from the date of arrival.



Spectrum of Chromeo™ 546 in PBS.



Immunofluorescence: HeLa cells stained with alpha Tubulin mAb (Catalog No. 39527) and the Chromeo 546 Goat anti-mouse secondary.

Protocol: Labeling Proteins with Chromeo™ 546 NHS-Ester

The protocol below is a guideline for using reactive Chromeo Dyes. As the total number and the surface exposition of free amines will be different for each protein to be labeled, we recommend that the labeling reaction be optimized for each target by using different molar ratios of protein to reactive-dye. In general, 1 mg of Chromeo 546 NHS-Ester can be used to label 15-25 mg of protein.

Preparation of the Working Dye Solution

Resuspend/dissolve 1 mg of Chromeo 546 NHS-Ester in 100 µl of DMF.

NOTE:

1. The dye-containing tube should be brought to room temperature before it is opened.
2. The Working Dye Solution should not be stored; it should be used within 1 hour of resuspension. For optimal yields of conjugated proteins, the dye should be resuspended immediately before starting the labeling procedure.

Preparation of the protein solution

Dissolve the appropriate amount of protein to be labeled in 1 ml of 50 mM Bicarbonate buffer, preferably at pH 8.3. The protein concentration should be 2-20 mg/ml.

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

Bicarbonate buffer, pH 8.3

Dissolve 2.1 g of NaHCO₃ in 500 ml doubly distilled water and adjust to pH 8.3 with 1 N NaOH or HCl.

Labeling reaction

1. While gently stirring the protein solution, add the appropriate volume of Working Dye Solution drop-wise into it. (Mix the protein solution carefully with a pipette tip while adding 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 20 µl of Working Dye Solution should be added. Due to variations in dye-protein 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 amine 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 546.

To calculate the DPR, measure the absorption of the conjugate at 280 nm (A_{280}) and the absorption of the dye at its maximum ($A_{546-max}$) by using a 1 cm path length cuvette.

$$DPR = C_{546}/C_{protein} \quad C: \text{ molar concentration}$$

$$C_{546} = A_{546-max}/\epsilon_{546-max} \quad \epsilon_{546-max}: \text{ molar extinction coefficient Chromeo 546 at its maximum} = 96,800 \text{ M}^{-1}\text{cm}^{-1}$$

$$C_{protein} = A_{protein}/\epsilon_{protein} \quad \epsilon_{protein}: \text{ molar extinction coefficient of the protein: } 200,000 \text{ M}^{-1}\text{cm}^{-1} \text{ for antibodies; } 43,800 \text{ M}^{-1}\text{cm}^{-1} \text{ for BSA; } 37,000 \text{ M}^{-1}\text{cm}^{-1} \text{ for lysozyme}$$

$$A_{protein} = A_{280} - (A_{546-max} \times \epsilon_{546-280}/\epsilon_{546-max}) \quad \epsilon_{546-280}: \text{ molar extinction coefficient Chromeo 546 at 280 nm} = 9,000 \text{ M}^{-1}\text{cm}^{-1}$$

Protocol (continued): Troubleshooting Guide for Chromeo™ 546 NHS-Ester Protein Labeling

Protein did not get labeled

1. The protein was in an incompatible buffer. The protein to be labeled cannot be in buffer that contain primary amines (like Tris or Glycine) or ammonium ions. Dialyze the protein into 1X PBS before performing conjugation.
2. The NHS-Ester has hydrolyzed and became non-reactive. Prepare the Working Dye Solution immediately before use and do not store any remaining solution.
3. The NHS-Ester was not resuspended/dissolved properly. Be sure to resuspend the lyophilized NHS-Ester in DMF when you prepare the Working Dye Solution.

Inefficient labeling

1. The protein being labeled was too dilute. Try to concentrate the starting material before conjugation.
2. The antibody or protein is in a buffer with a low pH. The optimal pH for labeling with NHS-Esters is between pH 8.1-8.3; try to reach this pH range by doubling the amount of Bicarbonate buffer or replace the buffer by dialysis or exchange.

Over-labeling of protein

1. There was not enough protein to label. Add more protein or reduce amount of reactive dye in your next conjugation.
2. The reaction time was too long. Reduce the amount of incubation time of the labeling reaction.