Chromeo™ 494 NHS-Ester

Catalog No: 15111, 16111

Format: 1 mg, 5 x 1 mg

Chemical Properties: Contents: 1 mg (Catalog No. 15111) or 5 x 1 mg (Catalog No. 16111) of lyophilized Chromeo™ 494 NHS-Ester
Net formula: $C_{30}H_{36}BrN_3O_6$; MW 614.54
Reagent color: light-red
Soluble in DMF, ethanol and methanol.

Fluorescent Properties: Chromeo 494 can be excited with a green laser. As the dye shows a large Stokes shift of 134 nm, the emission should be measured at a wavelength of 630 nm. Its fluorescent properties make Chromeo 494 an ideal partner for multiplexing with other 488-excitable dyes and conjugates.

Molar Extinction Coefficient: 55,000 $M^{-1}cm^{-1}$ (measured at A_{max})

Quantum Yield when conjugated to BSA: 28%

Excitation Wavelength: 494 nm

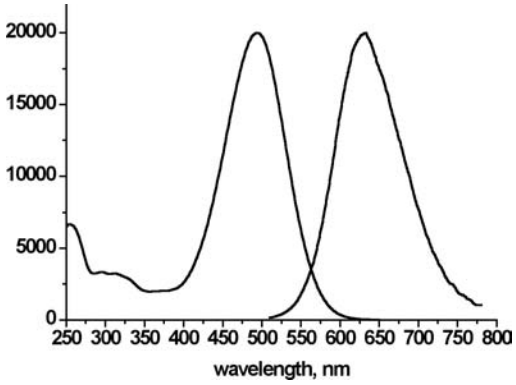
Emission Wavelength: 628 nm

Important product information:
NHS-activated dyes are very sensitive to moisture. They should be stored in the original vial 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.

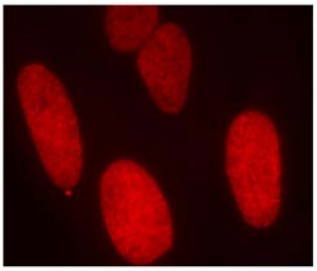
Prepare the dye-stock solution immediately before use. Do not store the stock-solution containing the activated dye.

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

Storage and Guarantee: To ensure stability, the lyophilized dye should be stored at -20ºC 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™ 494 in PBS.



Immunofluorescence: HeLa cells treated with etoposide stained with H2AX phospho Ser 139 rabbit antibody (Catalog No. 39117) and Chromeo 494 goat anti-rabbit secondary.

Protocol: Labeling Proteins with Chromeo™ 494 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 494 NHS-Ester can be used to label more than 30 mg of protein.

Preparation of the Working Dye Solution

Resuspend/dissolve 1 mg of Chromeo 494 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 $NaHCO_3$ 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 and 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 15 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 1.5 to 3.0 is optimal for conjugates with Chromeo 494.

To calculate the DPR, measure the absorption of the conjugate at 280 nm (A_{280}) and the absorption of the dye at its maximum ($A_{494\text{-}max}$) by using a 1 cm path length cuvette.

$DPR = C_{494}/C_{protein}$ C: molar concentration

$C_{494} = A_{494\text{-}max}/\varepsilon_{494\text{-}max}$ $\varepsilon_{494\text{-}max}$: molar extinction coefficient Chromeo 494 at its maximum = 55,000 $M^{-1}cm^{-1}$

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

$A_{protein} = A_{280} - (A_{494\text{-}max} \times \varepsilon_{494\text{-}280}/\varepsilon_{494\text{-}max})$ $\varepsilon_{494\text{-}280}$: molar extinction coefficient Chromeo 494 at 280 nm = 6,200 $M^{-1}cm^{-1}$

Protocol (continued): Troubleshooting Guide for Chromeo™ 494 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.

Chromeo™ 494 conjugates in fluorescent microscopy

To ensure a maximum in photostability of Chromeo 494 conjugates under all experimental conditions, we recommend the use of TDE or Mowiol as mounting media in fluorescence microscopy experiments.

TDE (2,2-thiodiethanol) or Mowiol mounting medium provide optimal fluorescent stability and inhibits photobleaching during examination by traditional and super-resolution microscopy. The use of Vectashield and MAXfluor Mounting Medium are not recommended as they might negatively influence the stability of Chromeo 494.