

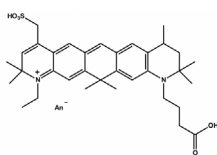
07376 Atto 647 NHS ester

Application

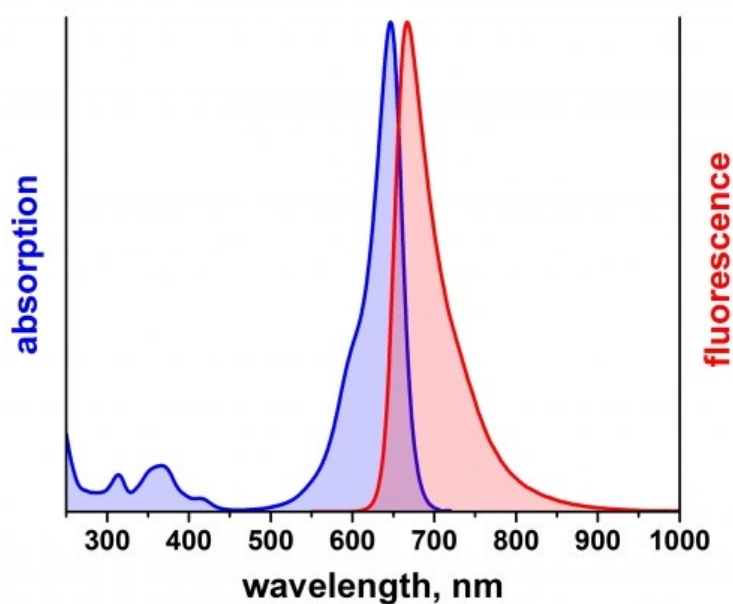
Atto 647 belongs to a new generation of fluorescent labels for the red spectral region. The dye is designed for application in the area of life science, e.g. labeling of DNA, RNA or proteins. Characteristic features of the label are strong absorption, high fluorescence quantum yield, high photostability, good water solubility, and very little triplet formation. Atto 647 is a zwitterionic dye with a net electrical charge of zero. Atto 647 is a pH sensitive product. While practically stable up to pH 8, it slowly degrades at higher pH.

Product Description

MW	811 g/mol
λ_{abs}	647 nm
ϵ_{max}	$1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$
λ_{fl}	667 nm
η_{fl}	20 %
τ_{fl}	2.4 ns
CF ₂₆₀	0.08
CF ₂₈₀	0.04



Optical data of the carboxy derivative (in aqueous solution)



Storage: Store at -20°C and protected from light.



Directions for labeling of proteins with Atto 647 NHS ester

Dissolve protein in of bicarbonate buffer (0.1 M, preferably of pH 8.3) at 2 mg/ml. Concentrations below 2 mg protein per ml will decrease labeling efficiency. Protein or peptide solutions must be free of any amine-containing substances such as Tris, glycine or ammonium salts. Antibodies in solutions of Tris buffers may be dialyzed against 10-20 mM PBS. The desired pH can be obtained by adding 0.1 ml of 1 M sodium bicarbonate buffer, pH 8.3, for each ml of dialyzed antibody solution.

Dissolve Atto 647 NHS ester in amine-free, dry DMF or DMSO at 2 mg/ml (e.g. 1 mg Atto 647 NHS in 500 μ l). This solution should always be prepared immediately before conjugation.

As number and position of amine groups vary between different proteins, the optimum of dye/protein ratio also varies. Wherefore we recommend, to try out different ratios when labeling a certain protein for the first time.

In general a molar ratio of 1-2 may be suitable for smaller proteins, much higher ratios for large proteins. To obtain a ratio in this range, add a twofold molar excess of reactive dye to the protein solution. For an antibody, a ratio of 8 or higher may be a good starting point. For such ratio, add 50 μ l of dye solution to 1 ml protein solution. Variations due to different reactivities of both the protein (number, position and reactivity of amine groups) may occur. This may necessitate individual optimization of the dye to protein ratio used in the reaction in order to optimize the results.

Incubate the reaction at room temperature for 30 to 60 min under constant or repeated stirring.

Separation of labeled proteins

The labeled protein can be separated from unreacted dye by gel permeation chromatography, e.g. using a Sephadex™ G-25, G50 or Bio-Gel™ P-10 column. We recommend to use Sephadex™ G-25. The column should have a diameter of at least 1 cm and a length of 12 cm. It can be equilibrated with phosphate buffer of pH 7.2 (22 mM) or another buffer of choice. The same buffer can be used for elution. Usually, the first fluorescent band is the labelled protein, while free dye will elute in a second fluorescent band.

In case you have to work with diluted samples, you may purify the conjugate by extensive dialysis. But this is less efficient and not as fast as purification by gel filtration.

Storage of conjugates

In general, conjugates should be stored under the same conditions used for the unlabeled protein. For storage in solution at 4°C, 2 mM sodium azide can be added as a preservative. Typically, protein conjugates will be stable for several months. For long-term storage, aliquots may be frozen at -20°C to avoid repeated freezing and thawing. Protect from light. If your protein tends to instability please use one of our BioStab solutions specially designed for stabilization of proteins.

After long-term storage of conjugate solutions we recommend to centrifuge in a micro-centrifuge before use. This will remove any aggregates which might have formed.

Directions for oligo-nucleotides with Atto 647 NHS ester

Prepare a solution of 0.1 mM solution (e.g. 5 nmol in 50 μ l) of amino-modified oligo-nucleotide in carbonate buffer (0.2 M, pH 8-9). Prepare a solution of 5 mg/ml activated label in anhydrous DMF. Add ~ 50 μ l of oligo-nucleotide solution to 30 μ l of label solution. Incubate the reaction at room temperature for 2 hours under shaking. If longer reaction times are required, the pH value should be diminished to pH 7-7.5.

Separation of labeled oligo-nucleotides

Conjugated oligo-nucleotides can be separated from free dye using gel filtration or reversed phase HPLC.

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Precautions and Disclaimer

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