01464 Atto 633 NHS ester

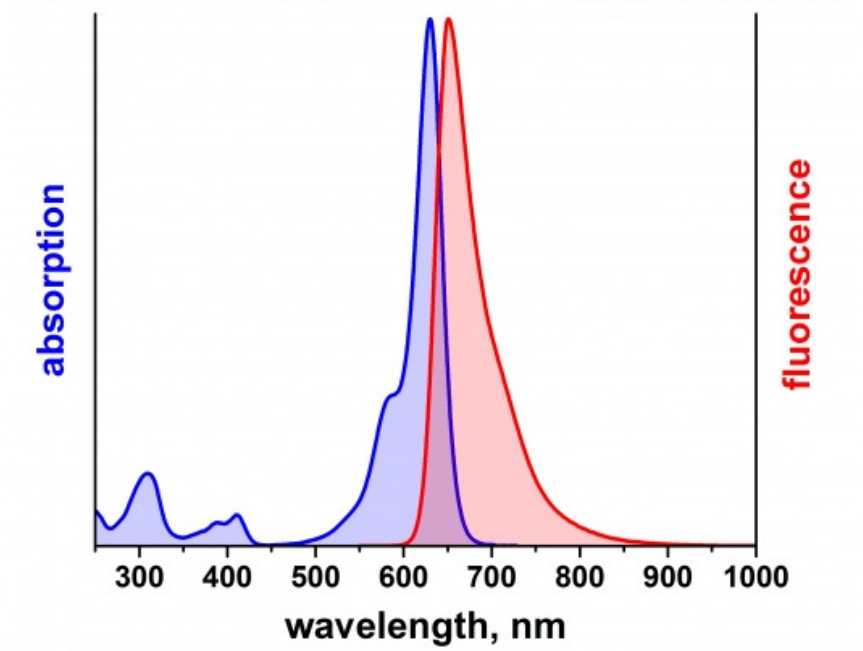
Application

Atto 633 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. labelling 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 633 is a cationic dye. After coupling to a substrate the dye carries a net electrical charge of $^{+1}$. In common with most Atto-labels, absorption and fluorescence are independent of pH, at least in the range of pH 2 to 11, used in typical applications.

Product Description

MW	749 g/mol (free acid)
λ_{abs}	630 nm
ε_{max}	1.3 x 10^5 M^{-1} cm^{-1}
λ_{fl}	651 nm
η_{fl}	64 %
τ_{fl}	3.3 ns
CF_{260}	0.04
CF_{280}	0.05

Optical data of the carboxy derivative (in aqueous solution)



Directions for labeling of proteins with Atto 633 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 633 NHS ester in amine-free, dry DMF or DMSO at 2 mg/ml (e.g. 1 mg Atto 633 in 500 µl). 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 ratio of 1-2 may be suitable. To obtain a ratio in this range, add a twofold molar excess of reactive dye to the protein solution. For large proteins, namely antibodies, we recommend a higher labelling ratio, using at least 4:1 molar excess of label, adding 30 µl of dye solution to 1 ml protein solution. You may further increase the intensity of fluorescence labelling by using molar excess of up to 15:1. The optimal ratio is dependent on the antibody labelled.
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 unstability 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 633 NHS ester
Prepare a solution of 0.1 mM solution (e.g. 5 nmol in 50 µl) 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 µl of oligo-nucleotide solution to 30 µl 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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