

72464 Atto 565 NHS ester

Application

Atto 565 is a fluorescent label belonging to the class of Rhodamine dyes. The dye is intended 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, and high thermal and photo-stability. Thus Atto 565 is highly suitable for single-molecule detection applications and high-resolution microscopy such as PALM, dSTORM, STED etc. Additionally the dye highly qualifies to be applied in flow cytometry (FACS), fluorescence in-situ hybridization (FISH) and many more. The dye is moderately hydrophilic. The fluorescence is excited most efficiently in the range 545 - 575 nm.

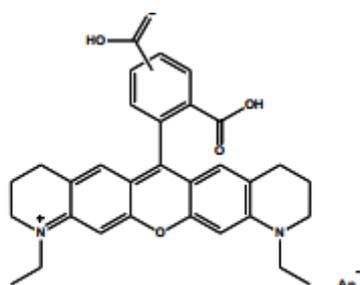
As supplied, Atto 565 consists of a mixture of two isomers with practically identical absorption and fluorescence.

Product Description

Net formula C₃₅H₃₄ClN₃O₁₁ (incl. counterion)
Solubility soluble in DMF, ethanol, methanol

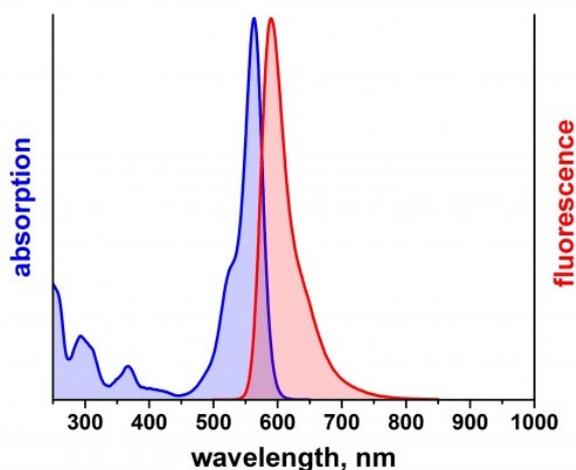
MW	708 g/mol
λ_{abs}	564 nm
ϵ_{max}	$1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$
λ_{fl}	590 nm
η_{fl}	90 %
τ_{fl}	4.0 ns
CF ₂₆₀	0.27
CF ₂₈₀	0.12

Optical data of the carboxy derivative (in aqueous solution)



Structure of free acid

Storage: protected from light at -20°C



Directions for labeling of proteins with Atto 565 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 565 NHS ester in amine-free, dry DMF or DMSO at 2 mg/ml (e.g. 1 mg Atto 565 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 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 an antibody, add 10 μ l of dye solution to 1 ml protein solution. 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 565 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

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

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