93516 Atto Rho6G NHS ester

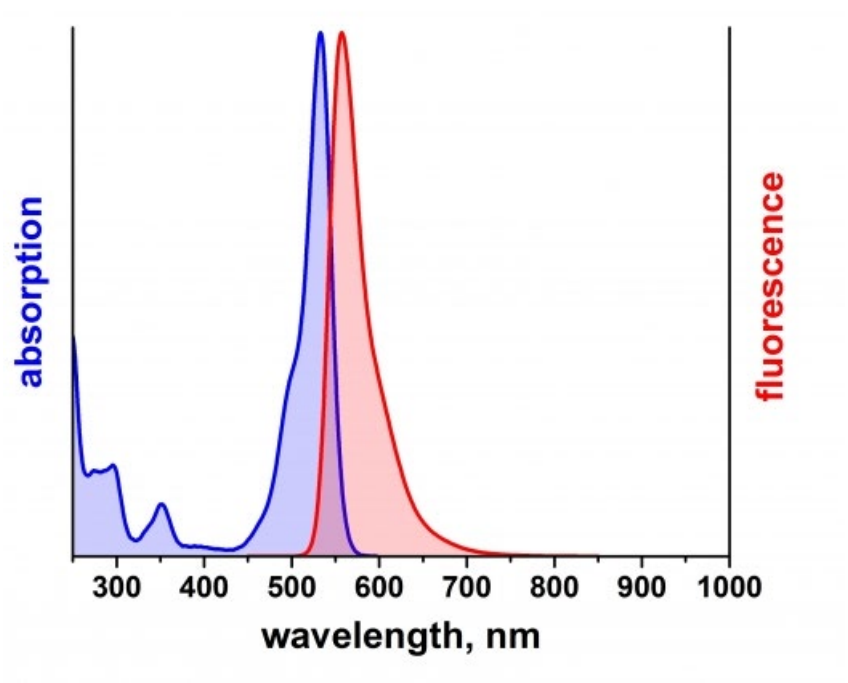
Application

Atto Rho6G is a new rhodamine dye, based on the well-known laser dye Rhodamine 6G. The new label is functionalized for coupling to biomolecules such as DNA, RNA or proteins. Atto Rho6G shows strong absorption, extraordinary high fluorescence quantum yield, high thermal and photo-stability, and very little triplet formation. The dye is highly suitable for single-molecule detection applications and high-resolution microscopy.
After coupling to a substrate Atto Rho6G carries a net electric charge of $^{+1}$. The label is moderately hydrophilic.

Product Description

MW	711 g/mol
λ_{abs}	533 nm
ε_{max}	1.15 x 10^5 M^{-1} cm^{-1}
λ_{fl}	557 nm
η_{fl}	90 %
τ_{fl}	4.1 ns
CF_{260}	0.19
CF_{280}	0.16

Optical data of the carboxy derivative (in aqueous solution)



Storage: store at ≤ -20°C. Protect from long-term exposure to moisture and light.

Directions for labeling of proteins with Atto Rho6G 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 Rho6G NHS ester in amine-free, dry DMF or DMSO at 2 mg/ml (e.g. 1 mg Atto Rho6G NHS 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 an antibody, add 10 µl 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 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 Rho6G 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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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.