81062 Atto 514 maleimide

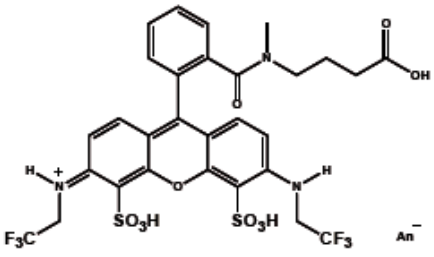
Application

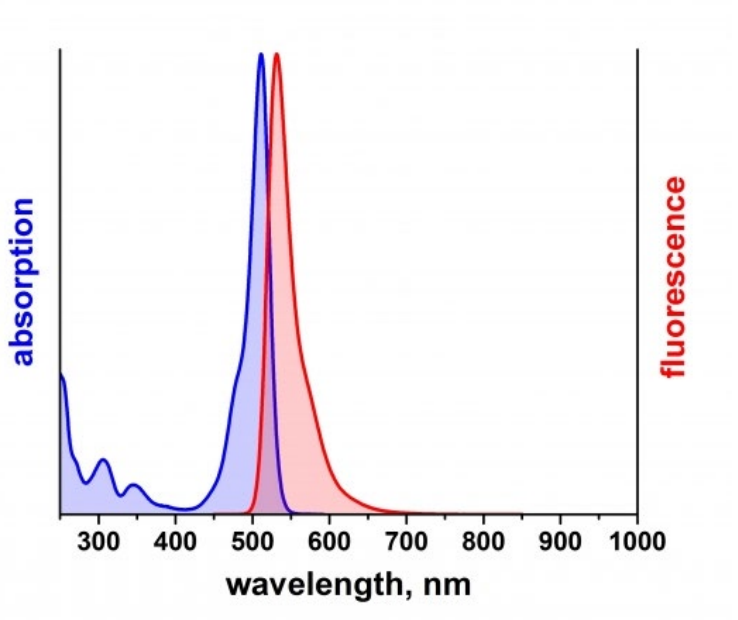
Atto 514 is a new hydrophilic fluorescent label with excellent water solubility. The dye exhibits strong absorption, high fluorescence quantum yield and exceptional thermal and photo-stability. Thus Atto 514 is highly suitable for
single-molecule detection applications and high-resolution microscopy 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 fluorescence is excited most efficiently in the range 510 - 535 nm. A suitable source of excitation is the 514 nm line of the Argon-Ion laser.
The **maleimide** is suitable for labeling sulfhydryl (thiol) groups of proteins, in particular cystein residues.

Product Description

MW	990 g/mol
λ_{abs}	511 nm
ε_{max}	1.15 x 10^5 M^{-1} cm^{-1}
λ_{fl}	532 nm
η_{fl}	85 %
τ_{fl}	3.9 ns
CF_{260}	0.21
CF_{280}	0.07

Optical data of the carboxy derivative (in aqueous solution)





Maleimides are well suited for coupling to thiol groups. This is similar to iodacetamides, but maleimides do react more thiol selective. They do not show significant reaction with histidine or methionine. Hydrolysis of maleimides to a mixture of isomeric nonreactive maleamic acids can compete significantly with thiol modification, particularly above pH 8. Maleimides may be used for labelling of amines, which usually requires a higher pH than reaction of maleimides with thiols.
After coupling to a substrate the label carries a net electrical charge of $^{-1}$.

General procedure for labelling proteins with maleimides

1. Dissolve the protein at 50–100 µM in a suitable buffer at pH 7.0–7.5 at room temperature. Common buffers include 10–100 mM phosphate, Tris, HEPES. Under those conditions, the protein thiol groups are sufficiently nucleophilic so that they react almost exclusively with the reagent. Other protein amines mostly remain protonated and relatively unreactive.

2. Redue disulfide bonds in the protein. A 10-fold molar excess of a reducing agent such as DTT (43817) or TCEP (93284) is usually sufficient. If DTT is used, then dialysis is required to remove the excess DTT prior to introducing the reactive dye. This is not necessary for TCEP.

3. As thiols can be oxidized to disulfides, It may be advisable to carry out thiol modifications in an oxygen-free environment. This is particularly important if the protein has been treated with a reagent such as dithiothreitol prior to thiol modification. In this case, all buffers should be deoxygenated and the reactions carried out under an inert atmosphere to prevent reformation of disulfides.

4. Prepare a 10–20 mM stock solution of the reactive dye in a suitable solvent immediately prior to use (DMSO is the most common choice). Protect all stock solutions from light as much as possible by wrapping containers in aluminum foil.

5. Add sufficient protein-modification reagent from a stock solution to achieve an 10–20 molar excess compared to protein. Add the reagent dropwise to the protein solution as it is stirring.

6. Let the reaction proceed for 2 hours at room temperature or overnight at 4°C. In both cases reaction should take place in the dark.

7. Upon completion of the reaction with the protein, an excess soluble low molecular weight thiol (e.g. glutathione, mercaptoethanol) can be added to consume excess thiol-reactive reagent, thus ensuring that no reactive species are present during the purification step.

8. Separate the conjugate on a gel filtration column, such as a Sephadex™ G-25 column or equivalent matrix, or by extensive dialysis at 4°C in an appropriate buffer.

Storage: protected from light at -20°C

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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.