

68499 Atto 532 maleimide

Application

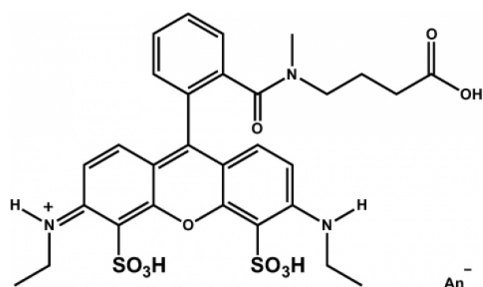
Atto 532 is a fluorescent label related to the well-known dye Rhodamine 6G. Characteristic features of the label are strong absorption, high fluorescence quantum yield, high photostability, and excellent water solubility. Thus Atto 532 is highly suitable for single-molecule detection applications and 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 515 - 545 nm.

A suitable excitation source for Atto 532 is the 532 nm output of the frequency-doubled Nd:YAG laser.

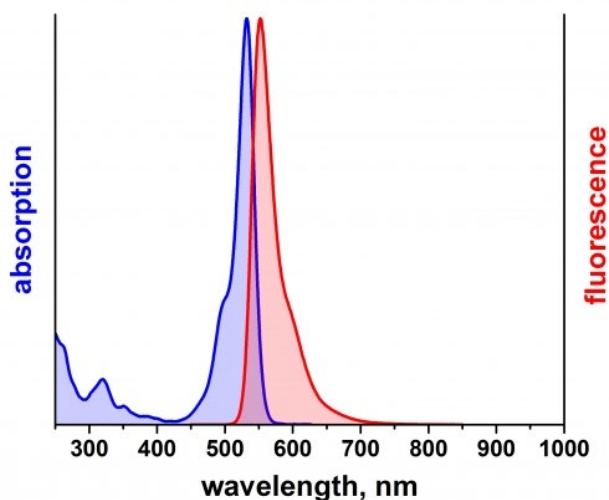
Product Description

MW	1063 g/mol
λ_{abs}	532 nm
ϵ_{max}	$1.15 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$
λ_{fl}	552 nm
η_{fl}	90 %
τ_{fl}	3.8 ns
CF ₂₆₀	0.20
CF ₂₈₀	0.09

Optical data of the carboxy derivative (in aqueous solution)



Structure of free acid



Storage: protected from light at -20°C



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)** Reduce 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.

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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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