

## 05316 Atto 647N maleimide

### Application

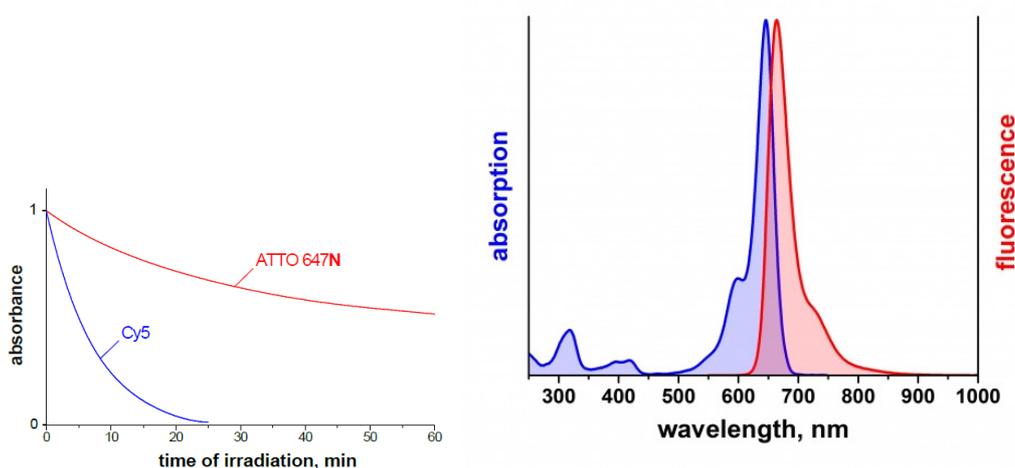
Atto 647N belongs to a new generation of fluorescent labels for the red spectral region. The dye is designed for application in the area of life science, e.g. labeling of DNA, RNA or proteins. Characteristic features of the label are strong absorption, excellent fluorescence quantum yield, high photostability, excellent ozone resistance, good solubility, and very little triplet formation. Atto 647N 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 in the range of 2 to 11, used in typical applications. As supplied Atto 647N consists of a mixture of two isomers with practically identical absorption and fluorescence properties.

### Product Description

MW	868 g/mol
$\lambda_{\text{abs}}$	646 nm
$\epsilon_{\text{max}}$	$1.5 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$
$\lambda_{\text{fl}}$	664 nm
$\eta_{\text{fl}}$	65 %
$\tau_{\text{fl}}$	3.5 ns
CF <sub>260</sub>	0.04
CF <sub>280</sub>	0.03

### Optical data of the carboxy derivative (in aqueous solution)



## General procedure for labelling proteins with maleimides

- 1)** Dissolve the protein at 50–100  $\mu\text{M}$  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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**Storage:** Store at –20°C and protected from light.

### 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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