

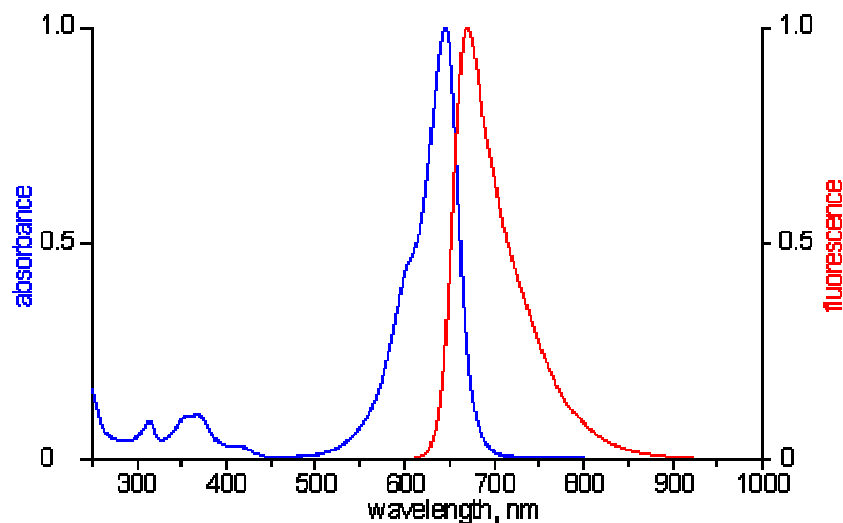
41784 Atto 647 maleimide

Application

Atto 647N is a new label for proteins and oligo-nucleotides with high molecular absorption (120.000) and quantum yield as well as sufficient stoke's shift (absorption maximum 645 nm, emission maximum 669 nm). Atto 647N is characterized by a high thermal and photostability. Absorption and fluorescence are independent of pH, at least in the most relevant range of pH 4 to 11. The dye is very stable under physiological pH-values and in buffers up to pH 8.5. However, it slowly degrades at higher pH. Atto 647 maleimide is supplied as solvent-free perchlorate salt and shows excellent solubility in polar solvents like DMF, DMSO or acetonitrile. After coupling to a substrate the dye moiety is electrically neutral.

Product Description

MW	832 g/mol
λ_{abs}	645 nm
ϵ_{max}	1.2×10^5 l / mol cm
λ_{fl}	669 nm
h_{fl}	20 %
τ_{fl}	2.3 ns
$CF = \epsilon_{280} / \epsilon_{\text{max}}$	0.04
Quantity	1 mg



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.

Storage Cooler, dark