Fluka

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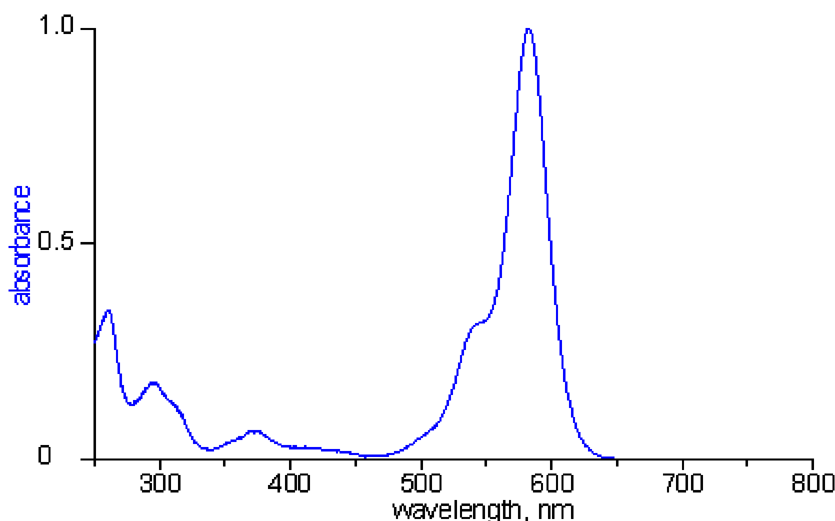
68152 Atto 580Q maleimide

Application
Atto 580Q is a new quencher for fluorescence characterized by high molecular absorption (110.000). Atto 580Q is characterized by a high photostability and thermostability. It is moderately hydrophilic, and soluble in polar solvents as DMF or DMSO. 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.

Product Description

ε	110.000
Abs. max.:	586nm(H_2O)
MW:	917
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) 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.

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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℃. 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℃ in an appropriate buffer.

Storage Cooler, dark