Product Specifications

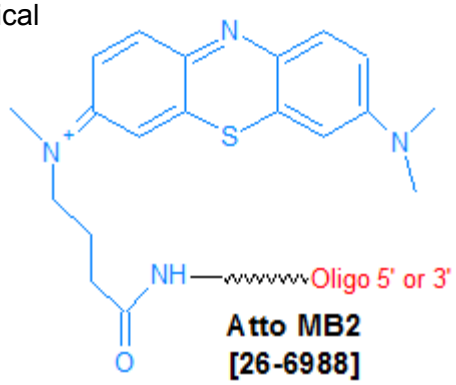
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2′-5′ linked Oligos

Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

Methylene Blue Azide

Category	Redox Electrochemical
Modification Code	MB-N3
Reference Catalog Number	26-6988
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	553



Atto MB2
[26-6988]

Methylene Blue Azide is a derivative of the well-known redox dye Methylene Blue. The azide derivative enables use in copper free click chemistry reactions with DBCO labelled reactants.
The dye can be reversibly reduced to the colorless leuko form. Upon oxidation (e.g. with oxygen) the dye recovers, and the absorption is fully restored.Conventional and popular dyes that are derivatives of fluoroscein (FAM, HEX and TET) and Cyanine dye derivatives (Cy3, Cy5, Cy5.5, Cy7 etc) are commonly used for fluorescently labeling oligos for use as molecular probes for real time PCR, FISH analysis and fragment analysis. For most purposes these provide a good range in wavelength and other optical properties and are available as amidites for direct coupling to oligos using automated chemistry. Other fluorescent dyes are available as N-hydroxysuccinimide (NHS) for conjugation using a primary amine group linked to the oligos. A new series of Atto dyes are now available that are are designed for high sensitivity applications, including single-molecule detection.
ATTO Dyes are a series of fluorescent labels and dyes manufactured by ATTO-TEC GmbH in Siegen, Germany. The ATTO Dye series covers a spectral range from 390 nm in the UV to 740 nm in the near infrared allowing excitation with most commonly used light sources. The dyes typically are derivatives of coumarins, rhodamines, carbopyronins and oxazines. Compared with other labels especially for the red region of the spectrum, ATTO-labels show excellent photostability and brightness. Atto labels have rigid structures that do not show any cis-trans isomerization. Thus these labels display exceptional intensity with minimal spectral shift on conjugation. The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of such dyes are ill-defined. ATTO-dyes have a molecular structure that ensures high rigidity of the chromophore. They do not form equilibria with various isomers, their optical properties are nearly independent of solvent and temperature. ATTO 647N fluoresces twice as strong as Cy5 in aqueous solution. In addition many common fluorescent labels especially cyanine dyes like Cy5 deteriorate even without any irradiation (in the dark), in particular when exposed to small concentrations of ozone present in the laboratory atmosphere.

Under identical conditions of ozone exposure the new dyes ATTO 633, ATTO 647N and ATTO 655 last up to 100 times longer than cyanines like Cy5 and Alexa Fluor 647. This is very important in microarray applications, where the dye molecules are located at the surface and thus are in direct contact with the atmosphere.

Copper-free Click Chemistry Modifications

Use azide modified oligos with DBCO Cyclooctyne-based modifications for ease of copper-free click reagents. These are simple to use and has excellent click performance in 17 hours or less at room temperature. Gene Link offers 5'-DBCO-TEG for preparing oligos with 5'-DBCO and a 15 tom triethylene glycol spacer arm, DBCO-dT for inserting a DBCO group at any position within the oligonucleotide and DBCO-sulfo-NHS Ester is also offered for post-synthesis conjugation reactions. DBCO-modified oligos may be conjugated with azides in organic solvents, such as DMSO, or aqeous buffers. Depending on the azide used, the reaction will go to completion in 4-17 hours at room temperature.

Azide C3 is available to introduce a stable azide group at the 3' of an oligo. Use Azide butyrate NHS [26-6922] for introduction of azide at internal or 5' position by conjugating to an amino-modified oligonucleotide. Introduction can be done at either the 5'- or 3'-end, or internally. To do this, the oligo first must be synthesized with a primary amino functional group modification, e.g Amino C6 for the 5' end or amino C7 for the 3' end for the ends) or the amino C6 version of the base phosphoramidite (for internal labeling). The Azidobutyrate NHS ester is then manually attached to the oligo through the amino group in a separate reaction post-synthesis. The presence of the azide allows the user to use "Click Chemistry" (a [3+2] cycloaddition reaction between alkynes and azides, using copper (I) iodide as a catalyst) to conjugate the azide-modified oligo to a terminal alkyne-modified oligo with extremely high regioselectivity and efficiency (1,2). Preparation of the alkyne-modified oligo can be achieved using the 5'-Hexynyl modifier (see its respective tech sheet for details). Click chemistry can be used to form short, cyclic oligos that can be used as research tools in various biophysical and biological studies (3). In particular, they have considerable potential for in vivo work, as cyclic oligos are known to be very stable in serum for up to several days.

References

1. Huisgen, R. *Angew. Chem. Int. Ed.* (1963), **2]**: 565-568.
2. Rostovtsev, V.V., Green, L.G., Fokin, V.V., Sharpless, K.B. A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective Ligation of Azides and Terminal Alkynes. *Angew. Chem. Int. Ed.* (2002), **41**: 2596-2599.
3. Kumar, R., El-Sagheer, A., Tumpane, J., Lincoln, P., Wilhelmsson, L.M., Brown, T. Template-Directed Oligonucleotide Strand Ligation, Covalent Intramolecular DNA Circularization and Catenation Using Click Chemistry. *J. Am. Chem. Soc.* (2007), **129**: 6859-6864.