



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.

Redox Electrochemical Introduction

Electrochemical sensors based on the target-induced folding or unfolding of electrode-bound oligonucleotides, including sensors for the detection of specific nucleic acids by hybridization and using aptamers for proteins and other small molecules including drugs and metabolites. These devices, which are often termed electrochemical DNA (E-DNA) and E-AB (electrochemical, aptamer-based) sensors, are comprised of an oligonucleotide probe modified with a redox reporter like ferrocene or methylene blue at one terminus and attached to a gold electrode via a thiol-gold bond at the other. Binding of an analyte to the oligonucleotide probe changes its structure and dynamics, which, in turn, influences the efficiency of electron transfer to the interrogating electrode. This class of sensors perform well even when challenged directly with blood serum, soil and other complex, multi-component sample matrices.

Gene Link also offers various modifications that can be used for conjugation to solid surfaces with either thiol, amino or carboxyl groups. Other bifunctional groups like EMCH are also available. Various fluorescent dyes can also be used in conjunction with redox dyes for signal detection using FRET.

Redox Electrochemical Design Protocols

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Redox Electrochemical Applications

Ferrocene-dT

Ferrocene-dT is a modified base nucleotide that contains a redox-active ferrocene moiety. Ferrocene is a sandwich compound composed of two cyclopentadienyl rings bound on opposite sides of a central iron atom (1). When incorporated into an oligonucleotide, the presence of ferrocene enables its use as an electrochemical (EC) probe for nucleic acid analysis. Ferrocene-modified probes can be designed to bind to either single- or double-stranded targets, and the resulting double- or triple-stranded probe-target complex is typically detected by HPLC with a standard electrochemical detector, with reported sensitivity at the sub-femtomole level (2,3). Ferrocene-modified probes covalently attached to a gold electrode surface have also been used in EC-based SNP assay, one probe to detect wild-type, and the other the SNP (4). In an alternative format, a “sandwich SNP assay” has also been studied. Here, a capture oligo was covalently bound to a gold surface via several phosphorothiolate linkages to capture the desired target DNA and hold it close to the gold surface. The targeted region for the capture oligo contains the SNP. A second, ferrocene-modified detection probe, hybridizes to a different, highly conserved, part of the target oligo to serve as the detector. If the target has been captured, electron transfer occurs between the ferrocene of the detection probe and the gold surface, producing an electrochemical signal (5). Ferrocene-modified DNA aptamers, designed to bind to one specific biochemical target molecule (DNA, RNA, proteins, etc.) have also been used to make aptamer-based EC sensors (6). EC probes also have significant potential as a low cost alternative to fluorescent-based probes in DNA microarray systems designed for use in clinical or medical diagnosis (7,8).

Methylene Blue

Methylene Blue (MB) NHS is a redox-active, heterocyclic aromatic dye that, when incorporated at the 5' or 3'-end of an oligonucleotide, enables the modified oligo's use as an electrochemical (EC) probe for nucleic acid analysis. Currently, there is considerable interest in using MB-modified oligonucleotides as aptamer probes for developing electrochemical DNA sensors for selective and sensitive detection of specific biochemical targets (DNA, RNA, proteins, etc.) in complex samples (for example, blood serum) (1,2). Such sensors are constructed by covalent attachment (typically through one or more thiol groups) of the MB-modified DNA probes to the surface of a gold electrode. The binding of target to probe leads to changes in the structural dynamics of the probe DNA that change the distance between the MB moiety and the gold surface. For “signal-on” sensors, the MB moiety moves close enough to the gold surface to cause electron transfer between the two, and generation of an electrochemical signal indicating presence of target in the sample (3). For “signal-off” sensors, the MB moiety moves away from the gold surface, halting electron transfer between the two, with the subsequent loss of an electrochemical signal indicating presence of target in the sample (4). Intensive work continues to move these systems beyond proof of principle and towards commercial availability.

References

Ferrocene References

1. Neto, F., Pelegrino, A., Caramori, A., Darin, V.A. Ferrocene: 50 Years of Transition Metal Organometallic Chemistry—From Organic and Inorganic to Supramolecular Chemistry. *ChemInform* (2004), **35**: no. doi: 10.1002/chin.200443242.
2. Takenaka, S., Uto, Y., Kondo, H., Ihara, T., Takagi, M. Electrochemically active DNA probes: Detection of target DNA sequences at femtomole level by high-performance liquid chromatography with electrochemical detection. *Anal. Biochem.* (1994), **218**: 436-443.
3. Ihara, T., Maruo, Y., Takenaka, S., Takagi, M. Ferrocene-oligonucleotide conjugates for electrochemical probing of DNA. *Nucleic Acids Res.* (1996), **24**: 4273-4280.
4. Yu, C.J., Wan, Y., Yowanto, H., Li, J., Tao, C., James, M.D., Tan, C.L., Blackburn, G.F., Meade, T.J. Electronic Detection of Single-Base Mismatches in DNA with Ferrocene-Modified Probes. *J. Am. Chem. Soc.* (2001), **123**: 11155-11161.
5. Nakayama, N., Ihara, T., Nakano, K., Maeda, M. DNA sensors using a ferrocene-oligonucleotide conjugate. *Talanta* (2002), **56**: 857-866.
6. Radi, A-E., Sanchez, J.L.A., Baldrich, E., O'Sullivan, C.K. Reagentless, Reusable, Ultrasensitive Electrochemical Molecular Beacon Aptasensor. *J. Am. Chem. Soc.* (2006), **128**: 117-124.
7. Liepold, P., Wieder, H., Hillebrandt, H., Friebel, A., Hartwich, G. DNA-arrays with electrical detection: A label-free low cost technology for routine use in life sciences and diagnostics. *Bioelectrochem.* (2005), **67**: 143-150.
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Methylene Blue References

1. Ricci, F., Lai, R.Y., Plaxco, K.W. Linear, redox modified DNA probes as electrochemical DNA sensors. *Chem. Comm.* (2007), **36**: 3768-3770.
2. Song, S., Wang, L., Li, J., Zhao, J., Fan, C. Aptamer-based biosensors. *Trends in Anal. Chem.* (2008), **27**: 108-117.
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Modification Code List

Modification	Code	Catalog Number
Anthraquinone-C2-dT	[AQ-dT]	26-6613
Ferrocene-dT	[Fc-dT]	26-6906
Methylene Blue Azide	[MB-N3]	26-6988
Methylene Blue Mal (Atto MB2-Mal)	[MB2-Mal]	26-6526
Methylene blue MB2-NHS	[MB2-N]	26-6908



Product Specifications

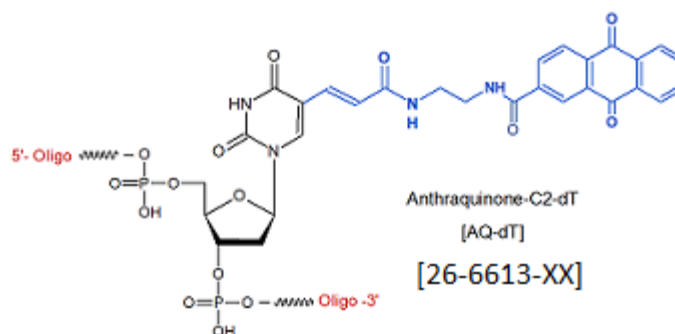
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Anthraquinone-C2-dT

Category	Redox Electrochemical
Modification Code	AQ-dT
Reference Catalog Number	26-6613
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	1077



Anthraquinone-modified oligonucleotides have proven to be versatile tools in stabilization of duplex DNA by intercalation (1), electrochemical detection of single-base mismatches (SNPs) (2), and as photoexcitable probes for the study of DNA hole transport (3). Charge-transfer phenomena in DNA either through oxidative or reductive pathways have received considerable attention in recent years due to their importance in biological environments such as protein-DNA complexes, DNA damage, mutations and cancer (4). Anthraquinones can be incorporated into oligonucleotides by using Anthraquinone-C2-dT during oligo synthesis. The anthraquinone moiety is useful for applications such as intercalation, duplex and triplex stabilization, photochemical immobilization, quenching of fluorescence, electrochemical detection, and charge transport through nucleic acids.

Anthraquinone derivatives as electron-acceptors Anthraquinone-C2-dT CEP features an electronically insulating tether that places the anthraquinone at a significant distance from the oligonucleotide. Dialkoxy derivatives of anthraquinone (AQ), dicyano-anthraquinone (DCAQ) and tetracyanoanthraquinone (TCAQ), displayed quasireversible, two sequential one-electron transfer redox reactions. DFT calculations of DCAQ and TCAQ demonstrate structural changes upon reduction, which is supported by spectroelectrochemical experiments. References H. Ihmels and D. Otto, *Top. Curr. Chem.*, 2005, 258, 161-204. Mikkel F. Jacobsen, Elena E. Ferapontova and Kurt V. Gothelf. *Org. Biomol. Chem.*, 2009, 905-908. A. Okamoto, T. Kamei and I. Saito, *J. Am. Chem. Soc.*, 2006, 128, 658-662. Review: H.-A. Wagenknecht, *Nat. Prod. Rep.*, 2006, 23, 973-1006 and references therein. Charge-transfer in DNA: From mechanism to Application, ed. H.-A. Wagenknecht, Wiley-VCH, Weinheim, 2005. E. Mayer-Enthart and H.-A. Wagenknecht, *Angew. Chem. Int. Ed.*, 1969, 8, 1228-1237. Murschell, A.E., Kan, W.H., Thangadurai, V. and Sutherland, T.C. *Phys. Chem. Chem. Phys.*, 2012, 14, 4626-4634.



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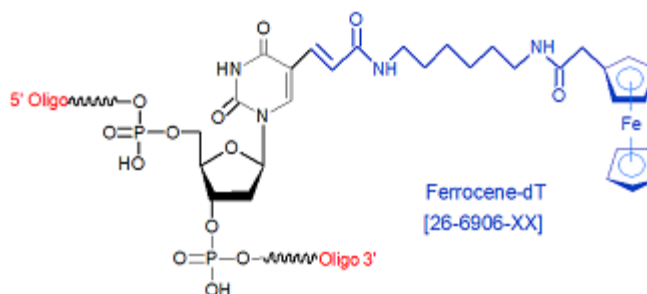
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Ferrocene-dT

Category	Redox Electrochemical
Modification Code	Fc-dT
Reference Catalog Number	26-6906
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	694.53



Ferrocene oligonucleotides should be stored under Argon and aqueous solutions should be degassed immediately. A convenient way to degas is the use of vacuum desiccator. We suggest making multiple small aliquots for storage at -20C or -80C for long term storage.

Ferrocene-dT is a modified base nucleotide that contains a redox-active ferrocene moiety. Ferrocene is a sandwich compound composed of two cyclopentadienyl rings bound on opposite sides of a central iron atom (1). When incorporated into an oligonucleotide, the presence of ferrocene enables its use as an electrochemical (EC) probe for nucleic acid analysis. Ferrocene-modified probes can be designed to bind to either single- or double-stranded targets, and the resulting double- or triple-stranded probe-target complex is typically detected by HPLC with a standard electrochemical detector, with reported sensitivity at the sub-femtomole level (2,3). Ferrocene-modified probes covalently attached to a gold electrode surface have also been used in EC-based SNP assay, one probe to detect wild-type, and the other the SNP (4). In an alternative format, a "sandwich SNP assay" has also been studied. Here, a capture oligo was covalently bound to a gold surface via several phosphorothiolate linkages to capture the desired target DNA and hold it close to the gold surface. The targeted region for the capture oligo contains the SNP. A second, ferrocene-modified detection probe, hybridizes to a different, highly conserved, part of the target oligo to serve as the detector. If the target has been captured, electron transfer occurs between the ferrocene of the detection probe and the gold surface, producing an electrochemical signal (5). Ferrocene-modified DNA aptamers, designed to bind to one specific biochemical target molecule (DNA, RNA, proteins, etc.) have also been used to make aptamer-based EC sensors (6). EC probes also have significant potential as a low cost alternative to fluorescent-based probes in DNA microarray systems designed for use in clinical or medical diagnosis (7,8). **References**

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Oligo Modifications

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Methylene Blue Azide

Category Redox Electrochemical

Modification Code MB-N3

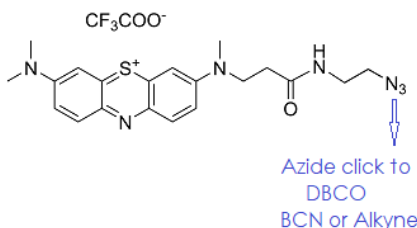
Reference Catalog Number 26-6988

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 553



Methylene Blue Azide Oligo
[26-6988-XX]

This modification is a post synthesis conjugation to BCN, alkyne or DBCO modification at the appropriate site for click conjugation. Gene Link offers post synthesis click free conjugation to oligos labelled with BCN at the 5' or 3' end. The azide group of Methylene Blue is linked to BCN group on the oligo. BCN group is required on the oligo. Additional charges applies for BCN

BCN-3

BCN-5

YIELD

Post synthesis conjugation modifications yields are lower as compared to direct automated coupling of modifications that are available as amidites. Approximate yield for various scales are given below.

~2 nmol final yield for 50 nmol scale synthesis.

~5 nmol final yield for 200 nmol scale synthesis.

~16 nmol final yield for 1 umol scale synthesis.

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 leuco form. Upon oxidation (e.g. with oxygen) the dye recovers, and the absorption is fully restored. Conventional and popular dyes that are derivatives of fluorescein (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 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 aqueous 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.
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Methylene Blue Maleimide (Atto MB2-Mal)

Category	Redox Electrochemical
Modification Code	MB2-Mal
Reference Catalog Number	26-6526
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	478

Methylene blue maleimide modification is a post synthesis conjugation to thiol group. The thiol group can be placed at the 5' and 3' and for internal positions a thiol dT C6 is used.

Methylene Blue (MB) NHS is a redox-active, heterocyclic aromatic dye that, when incorporated at the 5' or 3'-end of an oligonucleotide, enables the modified oligo's use as an electrochemical (EC) probe for nucleic acid analysis. Currently, there is considerable interest in using MB-modified oligonucleotides as aptamer probes for developing electrochemical DNA sensors for selective and sensitive detection of specific biochemical targets (DNA, RNA, proteins, etc.) in complex samples (for example, blood serum) (1,2). Such sensors are constructed by covalent attachment (typically through one or more thiol groups) of the MB-modified DNA probes to the surface of a gold electrode. The binding of target to probe leads to changes in the structural dynamics of the probe DNA that change the distance between the MB moiety and the gold surface. For "signal-on" sensors, the MB moiety moves close enough to the gold surface to cause electron transfer between the two, and generation of an electrochemical signal indicating presence of target in the sample (3). For "signal-off" sensors, the MB moiety moves away from the gold surface, halting electron transfer between the two, with the subsequent loss of an electrochemical signal indicating presence of target in the sample (4). Intensive work continues to move these systems beyond proof of principle and towards commercial availability.

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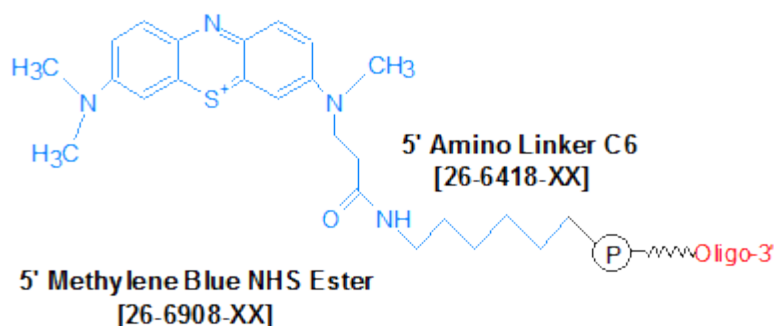
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Methylene blue MB2-NHS

Category	Redox Electrochemical
Modification Code	MB2-N
Reference Catalog Number	26-6908
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	338.4



[Click here for a list of other Redox Electrochemical Modifications.](#)

Methylene blue modification is a post synthesis conjugation to a primary amino group. The amino group can be placed at the 5' and 3' and for internal positions an amino modified base is used, e.g. **Amino dT C6 YIELD** NHS based modifications are post synthesis conjugation performed using a primary amino group. The yield is lower as compared to direct automated coupling of modifications that are available as amidites. Approximate yield for various scales are given below.

- ~2 nmol final yield for 50 nmol scale synthesis.
- ~5 nmol final yield for 200 nmol scale synthesis.
- ~16 nmol final yield for 1 umol scale synthesis.
- ~30 nmol final yield for 2 umol scale synthesis.
- ~75 nmol final yield for 5 umol scale synthesis.
- ~150 nmol final yield for 10 umol scale synthesis.
- ~225 nmol final yield for 15 umol scale synthesis.

Methylene Blue (e.g. Atto MB2) NHS is a redox-active, heterocyclic aromatic dye that, when incorporated at the 5' or 3'-end of an oligonucleotide, enables the modified oligo's use as an electrochemical (EC) probe for nucleic acid analysis. Currently, there is considerable interest in using MB-modified oligonucleotides as aptamer probes for developing electrochemical DNA sensors for selective and sensitive detection of specific biochemical targets (DNA, RNA, proteins, etc.) in complex samples (for example, blood serum) (1,2). Such sensors are constructed by covalent attachment (typically through one or more thiol groups) of the MB-modified DNA probes to the surface of a gold electrode. The binding of target to probe leads to changes in the structural dynamics of the probe DNA that change the distance between the MB moiety and the gold surface. For "signal-on" sensors, the MB moiety moves close enough to the gold surface to cause electron transfer between the two, and generation of an electrochemical signal indicating presence of target in the sample (3). For "signal-off" sensors, the MB moiety moves away from the gold surface, halting electron transfer between the two, with the subsequent loss of an electrochemical signal indicating presence of target in the sample (4). Intensive work continues to move these systems beyond proof of principle and towards commercial availability.

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