Affinity Ligands Introduction

Affinity ligands are molecules that are capable of binding with very high affinity to either a moiety specific for it or to an antibody raised against it. Examples include biotin (ligand)-streptavidin (moiety), digoxigenin (ligand)-anti-DIG-antibody and dinitrophenol (ligand)-anti-DNP-antibody. Incorporation of such ligands into an oligonucleotide, either at the ends or an internal base position, allows for the capture and purification of the oligo by affinity chromatography using the appropriate moiety/antibody as the capture medium. In addition, such ligand-labeled oligos can be detected using an appropriate indirect detection system. This allows the researcher to use affinity ligand-labeled oligos as highly sensitive and specific nucleic acid probes in solution-, membrane-, or bead-based assay systems.

Affinity Ligands Design Protocols

I. Indirect Detection of Targets (In situ hybridization (ISH))
A. Principle challenges of ISH:
The mRNA target in a tissue or cell is typically at a lower concentration than that used in blotting work. The mRNA target(s) may be masked by associated proteins. The mRNA target(s) may be sequestered within a cell or cellular structure.

Successful probing of a tissue or cells for a particular mRNA requires (a) increasing cell permeability, and (b) increasing the visibility of the target mRNA sequence to the probe while preserving the tissue or cell's structural integrity, and (c) to design the probe to achieve high resolution at high stringency. B. ISH protocols—things to consider:
1. Preparation of Biological Material:
Frozen tissue sections? Paraffin-embedded tissue sections? Cellular suspension?

Each format has its own particular advantages/disadvantages, and its own requirements for proper preparation. Always remember that since RNA is very sensitive to degradation, RNA handling protocols must be strictly observed. 2. Types of Probes/Advantages of Oligo Probes

Several different types of probes can be used, such as chemically synthesized DNA oligo probes (20-50 bases), ssDNA probes, dsDNA probes, and RNA probes (all 200-500 bases long and enzymatically synthesized). However, in most cases, well-designed, purified, chemically synthesized DNA oligo probes are not only cost-effective (both in materials and labor) and convenient to use, but provide a number of technical advantages. In particular, they are very stable and not subject to RNase degradation. they can be more specific than the longer probe types: can be designed to selectively recognize different members of closely related gene families by targeting the most variable DNA sequence within a gene family. they penetrate tissue better, due to their small size. they produce more reproducible results overall--every DNA oligo probe is identical. incorporation of affinity labels at the 5'- and 3'-ends does not interfere with hybridization to their respective targets. To avoid disruption of hybridization, internal labeling should be considered only for longer probes (30-50 bases).

3. Increasing Cell Permeability and mRNA target visibility

Increasing cell permeability involves treating the tissue or cells with some combination of (a) incubation in 0.2N HCl for 20-30 min (which extracts proteins from membranes), and (b) addition of Triton X-100 or SDS detergent (which extracts lipids from membranes). Detergent treatment is typically used for whole cell or frozen tissue sections. Increasing "visibility" of the mRNA target is accomplished by brief treatment of the tissue or cells with Proteinase K, which digests proteins that may be bound to the mRNA. 4. Pre-hybridization treatment to lower background staining

Because the DNA oligo probe contains affinity ligands for indirect detection, pre-hyb treatment of the tissue or cell sample is done to lower background staining. This is particularly important if the system used to visualize the probe-mRNA target complex utilizes enzymes such as peroxidase or alkaline phosphatase. To neutralize the endogenous presence of such enzymes in the sample, substances like 1% H_2O_2 (to block peroxidases) or levamisole (to block alkaline phosphatases) are added to the pre-hybridization solution. 5. Hybridization

For efficient hybridization of probe to mRNA target, hyb buffer typically includes the following components: volume/water excluder (e.g., dextran sulfate): absorbs water, reducing that available for dissolving DNA, which increases probe concentration, and hyb rates. organic solvents (e.g., formamide, DTT): reduce thermal stability of Watson-Crick bonds, allowing lower hyb temp. monovalent cations (e.g., NaCl): interact with phosphate backbone to decrease electrostatic repulsion between DNA strands, and increases hyb rates. EDTA: removes free divalent cations from solution, which strongly stabilize duplex DNA (undesirably increase hyb temp).

6. Recommended Controls

Poly dT/housekeeping gene probes--verifies tissue/tissue prep quality RNase treatment of tissue sections, then hybridization w/labeled anti-sense probe--verifies that probe is actually binding to mRNA Hybridize labeled sense and anti-sense probes in parallel to tissue sections--verifies probe binding is sequence-specific

Affinity Ligands Applications

For DNA-related applications, affinity ligands are used in two classes of applications, indirect detection of targets and affinity chromatography. For indirect detection, the ligand-labeled DNA probe is incubated with the target, the latter being either in solution or previously immobilized onto a solid phase (like a membrane). After binding of probe to target, the probe-target complex is treated with a dye- or enzyme-labeled detection moiety (for example, for biotin, a dye-labeled streptavidin (1); for Dig or DNP, an alkaline phosphatase-labeled-anti-Dig/DNP antibody, followed by incubation with a fluorogenic/colorimetric substrate (2)). In many cases, since probe length averages about 30 bases, incorporating three affinity labels, spaced about 15 bases apart to minimize steric hindrance, enables maximum potential sensitivity via indirect detection (3). Besides their importance as nucleic acid probes, ligand-labeled oligos are also useful for purification of cognate DNA molecules or DNA binding proteins by specific hybridization-based affinity chromatography (4). For example, biotinylated oligos can be bound to a streptavidin matrix, while Dig-labeled-oligos can be bound to an anti-Dig antibody matrix. For either case, the purification can be accomplished in a column, spin or magnetic bead format.

References

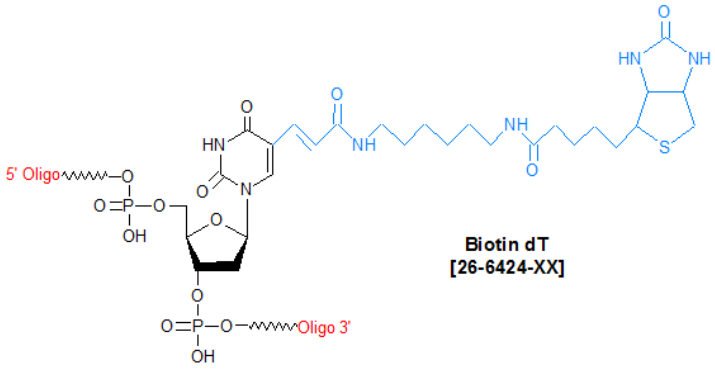
(1) Pinkel, D., Straume, T., Gray, J.W. Cytogenetic analyses using quantitative, high-sensitivity, fluorescence hybridization. Proc. Natl. Acad. Sci. USA (1986), 83: 2934-2938.
(2) Grzybowski, J., Will, D.W., Randall, R.E., Smith, C.A., Brown, T. Synthesis and antibody-mediated detection of oligonucleotides containing multiple 2,4-dinitrophenyl reporter groups. Nucleic Acids Res. (1993), 21: 1705-1712.
(3) Kessler, C., Holtke, H.J., Seibl, R., Burg, J., Muhlegger, K. Nonradioactive labeling and detection of nucleic-acids I: a novel DNA labeling and detection system based on digoxigenin-anti-digoxigenin ELISA principle (digoxigenin system). Biol. Chem. Hoppe-Seyler (1990), 371: 917-927.
(4) Bianconcini, A., Lupo, A., Capone, S., Quadro, L., et al. Transcriptional activity of the murine retinol binding protein gene is regulated by a multiprotein complex containing HMGA1, p54nrb/NonO, protein-associated splicing factor (PSF) and steroidogenic factor 1 (SF1)/liver receptor homologue 1 (LRH-1). Int. J. Biochem. Cell Biol. (2009), 41: 2189-2203.

Modificaton Code List

Modification	Code	Catalog Number
Biotin deoxythymidine dT	[Bio-dT]	26-6424
Dual Biotin	[Bio-Dual]	26-6421
Biotin NHS	[Bio-N]	26-6712
Biotin TEG (15 atom triethylene glycol spacer) 5'	[Bio-TEG-5]	26-6407F
Biotin-5'	[Bio-5]	26-6423
Desthiobiotin NHS	[DesBio-N]	26-6713
Desthiobiotin TEG	[DesBio-TEG]	26-6714
DesthiobiotinTEG Azide	[DesBioTEG-N3]	26-6725
Digoxigenin NHS	[Dig-N]	26-6429
DNP TEG (2, 4-dinitrophenyl)	[DNP-TEG]	26-6512
PC Biotin (photocleavable)	[PCBio]	26-6691

Biotin dT

Category	Affinity Ligands
Modification Code	Bio-dT
Reference Catalog Number	26-6424
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	684.7



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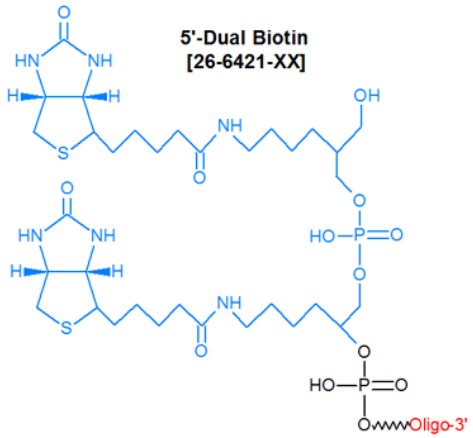
Biotin Dual

Category	Affinity Ligands
Modification Code	Bio-Dual
Reference Catalog Number	26-6421
5 Prime	Y
3 Prime	Y
Internal	N
Molecular Weight(mw)	874.8



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Biotin NHS

Category	Affinity Ligands
Modification Code	Bio-N
Reference Catalog Number	26-6712
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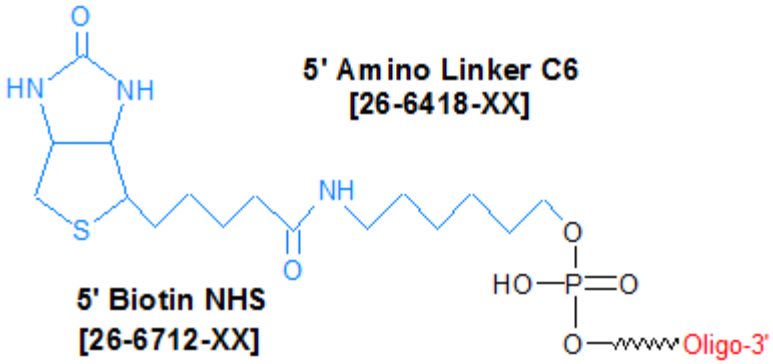
3 Prime	Y
Internal	Y
Molecular Weight(mw)	244.32



Click here for a list of other Affinity Ligand Modifications.

Biotin-NHS is an N-hydroxysuccinimide ester (NHS ester) of biotin. Biotin-NHS can be used to internally label an oligonucleotide with biotin at any base (that is, at a G, C, T or A position). To accomplish this, amino dG-C6/dC-C6/dA-C6/dT is first incorporated into the oligonucleotide, thereby placing an active primary amino group at the desired position. Biotin-NHS is then conjugated to the amino group in a separate reaction to form the final biotin-labeled product.

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.

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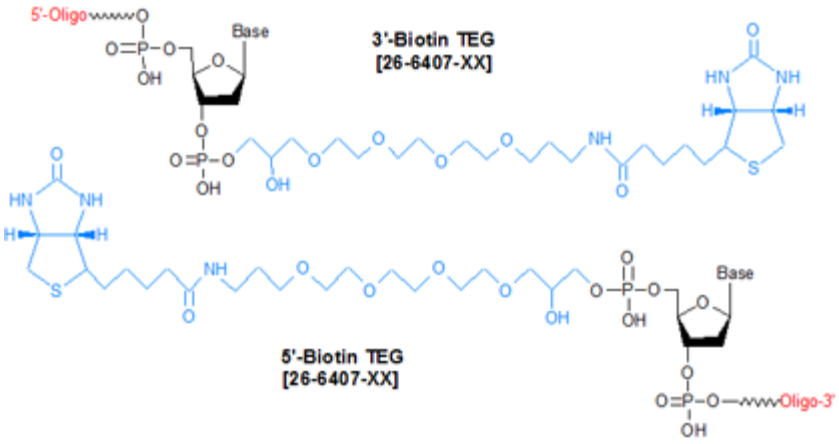
The primary amine labelled oligos can also be conjugated to carboxyl functional groups usually for solid supports applications using EDC mediated reaction as shown in the figure below.

References

1. Bengtstron, M., Jungell-nortamo, A., Syvanen, A-C. Biotinylation of Oligonucleotides Using a Water Soluble Biotin Ester.*Nucleos. Nucleot. Nucl.* (1990), **9**: 123-127.

Biotin TEG 5'

Category	Affinity Ligands
Modification Code	Bio-TEG-5
Reference Catalog Number	26-6407F
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	569.61



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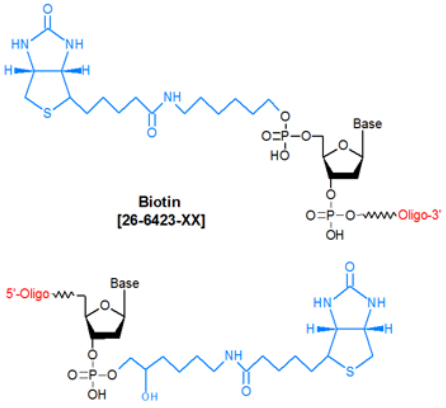
Oligo Modifications

Biotin-5'

Category	Affinity Ligands
Modification Code	Bio-5
Reference Catalog Number	26-6423
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	437.4



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Besides their importance as nucleic acid probes, biotinylated oligonucleotides are also useful for the purification of DNA binding proteins. In this context, the biotinylated oligonucleotide can be bound to a streptavidin matrix and used for either column or spin chromatography. For isolation of DNA binding proteins, the streptavidin-biotin-oligonucleotide complex is incubated with a crude cell extract containing nuclear proteins. Following appropriate washes, the proteins that bind selectively to the oligonucleotide sequence can be eluted under conditions that disrupt the protein:DNA complex. Because the binding of biotin to streptavidin is essentially irreversible and is resistant to chaotropic agents and extremes of pH and ionic strength, the elution conditions can be relatively stringent. Dual Biotin

Dual Biotin modification specifically can be used to add multiple biotin moieties at the 5'- or 3'-end of an oligo. The most common use of this modification is to incorporate two biotin (Dual Biotin) molecules in sequence (separated by a six-carbon linker) at the 5'-end of an oligo. This "Dual Biotin" has higher binding affinity for streptavidin than that of a single biotin. The additional binding strength can be critical for applications requiring the use of biotinylated DNA attached to streptavidin-coated beads at higher temperature (for example, in PCR). Dual Biotin is known to prevent or effectively reduce loss of biotinylated DNA from such beads during heating (1). Dual biotin also is used to label the linker primers in Serial Analysis of Gene Expression (SAGE) protocols (2).

For direct biotin-labeling of target RNA transcripts for microarray analysis, a special 3'-biotinylated donor nucleotide molecule containing three biotin molecules in sequence was synthesized and then ligated to the target RNA using T4 RNA ligase. The attachment of three biotins to RNA in this manner resulted in a 30% increase in target signal intensity and improved transcript detection sensitivity (3) . Multi Biotin

Multi-Biotin is a modification that can be added sequentially as many units as desired based on the application. Use Dual Biotin modification code [Bio-Dual] that is specific for two units of biotin.

For direct biotin-labeling of target RNA transcripts for microarray analysis, a special 3'-biotinylated donor nucleotide molecule containing three biotin molecules in sequence was synthesized and then ligated to the target RNA using T4 RNA ligase. The attachment of three biotins to RNA in this manner resulted in a 30% increase in target signal intensity and improved transcript detection sensitivity (3) . Desthiobiotin

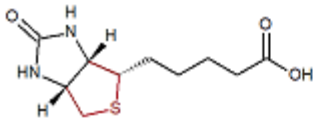
Desthiobiotin is a biotin derivative. Like biotin, desthiobiotin binds to streptavidin, but its binding affinity is considerably less (2x10E-9 M) than that of biotin (4.0x10E-14 M) (1). Consequently, oligonucleotides labeled with desthiobiotin can be easily displaced from streptavidin by biotin, thereby making recovery of the labeled oligo (for example, in affinity purification protocols) from a streptavidin-coated support a relatively simple process (2). Desthiobiotin-labeled oligos can also be conveniently eluted from streptavidin-coated supports by incubation in distilled water at 95C for 10 minutes (3). Gene Link recommends substitution of of desthiobiotin for biotin for those cases where reversible capture of oligonucleotides is desirable. Note that since desthiobiotin is in the form of an NHS ester, an active primary amino group (such as Amino Linker C6) must first be incorporated into the oligonucleotide, to allow for subsequent conjugation to desthiobiotin NHS ester.

Desthiobiotin NHS modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6. References

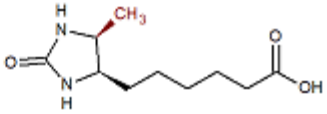
1. Huisgen, R. *Angew. Chem. Int. Ed.* (1963), **2**: 565-568.
2. Rostovtsev, V.V., Green, L.G., Fokin, V.V., Sharpless, K.B. A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective Ligation of Azides and Terminal Alkynes. *Angew. Chem. Int. Ed.* (2002), **41**: 2596-2599.
3. Green, N.M. Spectrophotometric determination of avidin and biotin. *Methods Enzymol.* (1970), **18A**: 418-424.
4. Hirsch, J.D., Eslamizar, L., Filanoski, B.J., Malekzadeh, N., Haugland, R.P., Beechem, J.M., Haugland, R.P. Easily reversible desthiobiotin binding to streptavidin, avidin, and other biotin-binding proteins: uses for protein labeling, detection, and isolation. *Anal. Biochem.* (2002), **308**: 343-357.
5. van Doom, R., Slawiak, M., Szemes, M., Dullemans, A.M., Bonants, P., Kowalchuk, G.A., Schoen, C.D. Robust Definition and Identification of Multiple Oomycetes and Fungi in Environmental Samples by Using a Novel Cleavable Padlock Probe-Based Ligation Detection Assay.*Appl. Environ. Microbiol.* (2009), **75**: 4185-4193.

Desthiobiotin NHS

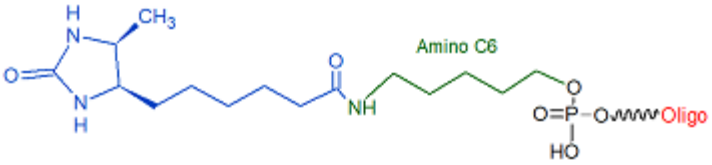
Category	Affinity Ligands
Modification Code	DesBio-N
Reference Catalog Number	26-6713
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	214.26



Biotin CAS: 58-85-5



Desthiobiotin CAS: 533-48-2



Desthiobiotin Oligo (NHS)
[26-6713-XX]

Click here for a list of other Affinity Ligand Modifications.

Desthiobiotin modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino 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
~160 nmol final yield for 10 umol scale synthesis
~240 nmol final yield for 15 umol scale synthesis

Desthiobiotin NHS & Desthiobiotin TEG

Desthiobiotin is a biotin derivative. Like biotin, desthiobiotin binds to streptavidin, but its binding affinity is considerably less (2x10E-9 M) than that of biotin (4.0x10E-14 M) (1). Consequently, oligonucleotides labeled with desthiobiotin can be easily displaced from streptavidin by biotin, thereby making recovery of the labeled oligo (for example, in affinity purification protocols) from a streptavidin-coated support a relatively simple process (2). Desthiobiotin-labeled oligos can also be conveniently eluted from streptavidin-coated supports by incubation in distilled water at 95C for 10 minutes (3). Gene Link recommends substitution of of desthiobiotin for biotin for those cases where reversible capture of oligonucleotides is desirable.
Desthiobiotin TEG is incorporated directly in an oligo and is available for 5' and 3'. Incorporation is also possible internally but it will break the nucleic acid base chain.

. Note that since Desthiobiotin NHS is in the form of an NHS ester, an active primary amino group (such as Amino Linker C6) must first be incorporated into the oligonucleotide, to allow for subsequent conjugation to desthiobiotin NHS ester.. Biotin

Biotin is an affinity label that can be incorporated at either the 5'- or 3'-end of an oligonucleotide, or at an internal position using biotin dT or Amino bases for conjugation to biotin-NHS.

Biotin has a high affinity for the bacterial protein, streptavidin, which can be conjugated to a solid support (such as magnetic beads) for use as a capture and immobilization medium for a biotinylated oligo. In the biotin phosphoramidite, the biotin is attached to a long spacer arm, which acts to minimize steric hindrance between the biotin moiety and the oligo, thereby providing streptavidin easy access to the biotin. Biotinylated oligos are most commonly used as probes or primers in a variety of in vitro and in vivo applications.

Besides their importance as nucleic acid probes, biotinylated oligonucleotides are also useful for the purification of DNA binding proteins. In this context, the biotinylated oligonucleotide can be bound to a streptavidin matrix and used for either column or spin chromatography. For isolation of DNA binding proteins, the streptavidin-biotin-oligonucleotide complex is incubated with a crude cell extract containing nuclear proteins. Following appropriate washes, the proteins that bind selectively to the oligonucleotide sequence can be eluted under conditions that disrupt the protein:DNA complex. Because the binding of biotin to streptavidin is essentially irreversible and is resistant to chaotropic agents and extremes of pH and ionic strength, the elution conditions can be relatively stringent. Dual Biotin

Dual Biotin modification specifically can be used to add multiple biotin moieties at the 5'- or 3'-end of an oligo. The most common use of this modification is to incorporate two biotin (Dual Biotin) molecules in sequence (separated by a six-carbon linker) at the 5'-end of an oligo. This "Dual Biotin" has higher binding affinity for streptavidin than that of a single biotin. The additional binding strength can be critical for applications requiring the use of biotinylated DNA attached to streptavidin-coated beads at higher temperature (for example, in PCR). Dual Biotin is known to prevent or effectively reduce loss of biotinylated DNA from such beads during heating (1). Dual biotin also is used to label the linker primers in Serial Analysis of Gene Expression (SAGE) protocols (2).

For direct biotin-labeling of target RNA transcripts for microarray analysis, a special 3'-biotinylated donor nucleotide molecule containing three biotin molecules in sequence was synthesized and then ligated to the target RNA using T4 RNA ligase. The attachment of three biotins to RNA in this manner resulted in a 30% increase in target signal intensity and improved transcript detection sensitivity (3) . Multi Biotin

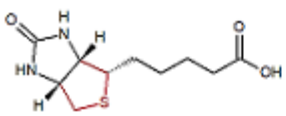
Multi-Biotin is a modification that can be added sequentially as many units as desired based on the application. Use Dual Biotin modification code [Bio-Dual] that is specific for two units of biotin.

For direct biotin-labeling of target RNA transcripts for microarray analysis, a special 3'-biotinylated donor nucleotide molecule containing three biotin molecules in sequence was synthesized and then ligated to the target RNA using T4 RNA ligase. The attachment of three biotins to RNA in this manner resulted in a 30% increase in target signal intensity and improved transcript detection sensitivity (3) . References

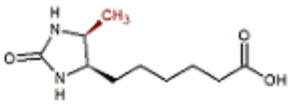
1. Huisgen, R. *Angew. Chem. Int. Ed.* (1963), **2**: 565-568.
2. Rostovtsev, V.V., Green, L.G., Fokin, V.V., Sharpless, K.B. A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective Ligation of Azides and Terminal Alkynes. *Angew. Chem. Int. Ed.* (2002), **41**: 2596-2599.
3. Green, N.M. Spectrophotometric determination of avidin and biotin. *Methods Enzymol.* (1970), **18A**: 418-424.
4. Hirsch, J.D., Eslamizar, L., Filanoski, B.J., Malekzadeh, N., Haugland, R.P., Beechem, J.M., Haugland, R.P. Easily reversible desthiobiotin binding to streptavidin, avidin, and other biotin-binding proteins: uses for protein labeling, detection, and isolation. *Anal. Biochem.* (2002), **308**: 343-357.
5. van Doom, R., Slawiak, M., Szemes, M., Dullemans, A.M., Bonants, P., Kowalchuk, G.A., Schoen, C.D. Robust Definition and Identification of Multiple Oomycetes and Fungi in Environmental Samples by Using a Novel Cleavable Padlock Probe-Based Ligation Detection Assay.*Appl. Environ. Microbiol.* (2009), **75**: 4185-4193.

Desthiobiotin TEG

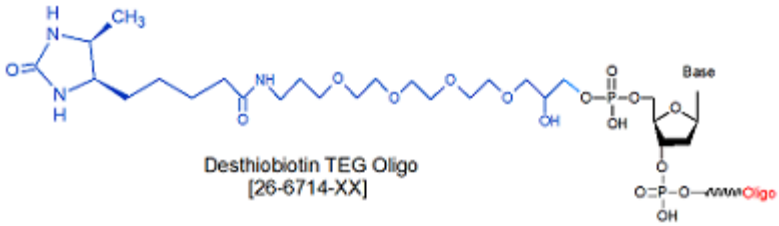
Category	Affinity Ligands
Modification Code	DesBio-TEG
Reference Catalog Number	26-6714
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	539.56



Biotin CAS: 58-85-5



Desthiobiotin CAS: 533-48-2



Desthiobiotin TEG Oligo
[26-6714-XX]

Click here for a list of other Affinity Ligand Modifications.
Desthiobiotin

Desthiobiotin is a biotin derivative. Like biotin, desthiobiotin binds to streptavidin, but its binding affinity is considerably less (2x10E-9 M) than that of biotin (4.0x10E-14 M) (1). Consequently, oligonucleotides labeled with desthiobiotin can be easily displaced from streptavidin by biotin, thereby making recovery of the labeled oligo (for example, in affinity purification protocols) from a streptavidin-coated support a relatively simple process (2). Desthiobiotin-labeled oligos can also be conveniently eluted from streptavidin-coated supports by incubation in distilled water at 95C for 10 minutes (3). Gene Link recommends substitution of of desthiobiotin for biotin for those cases where reversible capture of oligonucleotides is desirable. Note that since desthiobiotin is in the form of an NHS ester, an active primary amino group (such as Amino Linker C6) must first be incorporated into the oligonucleotide, to allow for subsequent conjugation to desthiobiotin NHS ester. Desthiobiotin TEG is incorporated directly in an oligo and is available for 5' and 3'. Incorporation is also possible internally but it will break the nucleic acid base chain. Biotin

Biotin is an affinity label that can be incorporated at either the 5'- or 3'-end of an oligonucleotide, or at an internal position using biotin dT or Amino bases for conjugation to biotin-NHS. Biotin has a high affinity for the bacterial protein, streptavidin, which can be conjugated to a solid support (such as magnetic beads) for use as a capture and immobilization medium for a biotinylated oligo. In the biotin phosphoramidite, the biotin is attached to a long spacer arm, which acts to minimize steric hindrance between the biotin moiety and the oligo, thereby providing streptavidin easy access to the biotin. Biotinylated oligos are most commonly used as probes or primers in a variety of in vitro and in vivo applications.

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Following appropriate washes, the proteins that bind selectively to the oligonucleotide sequence can be eluted under conditions that disrupt the protein:DNA complex. Because the binding of biotin to streptavidin is essentially irreversible and is resistant to chaotropic agents and extremes of pH and ionic strength, the elution conditions can be relatively stringent. Dual Biotin

Dual Biotin modification specifically can be used to add multiple biotin moieties at the 5'- or 3'-end of an oligo. The most common use of this modification is to incorporate two biotin (Dual Biotin) molecules in sequence (separated by a six-carbon linker) at the 5'-end of an oligo. This "Dual Biotin" has higher binding affinity for streptavidin than that of a single biotin. The additional binding strength can be critical for applications requiring the use of biotinylated DNA attached to streptavidin-coated beads at higher temperature (for example, in PCR). Dual Biotin is known to prevent or effectively reduce loss of biotinylated DNA from such beads during heating (1). Dual biotin also is used to label the linker primers in Serial Analysis of Gene Expression (SAGE) protocols (2).

For direct biotin-labeling of target RNA transcripts for microarray analysis, a special 3'-biotinylated donor nucleotide molecule containing three biotin molecules in sequence was synthesized and then ligated to the target RNA using T4 RNA ligase. The attachment of three biotins to RNA in this manner resulted in a 30% increase in target signal intensity and improved transcript detection sensitivity (3) . Multi Biotin

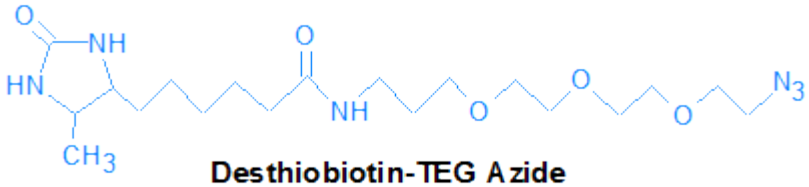
Multi-Biotin is a modification that can be added sequentially as many units as desired based on the application. Use Dual Biotin modification code [Bio-Dual] that is specific for two units of biotin.

For direct biotin-labeling of target RNA transcripts for microarray analysis, a special 3'-biotinylated donor nucleotide molecule containing three biotin molecules in sequence was synthesized and then ligated to the target RNA using T4 RNA ligase. The attachment of three biotins to RNA in this manner resulted in a 30% increase in target signal intensity and improved transcript detection sensitivity (3) . 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. Green, N.M. Spectrophotometric determination of avidin and biotin. *Methods Enzymol.* (1970), **18A**: 418-424.
4. Hirsch, J.D., Eslamizar, L., Filanoski, B.J., Malekzadeh, N., Haugland, R.P., Beechem, J.M., Haugland, R.P. Easily reversible desthiobiotin binding to streptavidin, avidin, and other biotin-binding proteins: uses for protein labeling, detection, and isolation. *Anal. Biochem.* (2002), **308**: 343-357.
5. van Doom, R., Slawiak, M., Szemes, M., Dullemans, A.M., Bonants, P., Kowalchuk, G.A., Schoen, C.D. Robust Definition and Identification of Multiple Oomycetes and Fungi in Environmental Samples by Using a Novel Cleavable Padlock Probe-Based Ligation Detection Assay.*Appl. Environ. Microbiol.* (2009), **75**: 4185-4193.

DesthiobiotinTEG Azide

Category	Click Chemistry
Modification Code	DesBioTEG-N3
Reference Catalog Number	26-6725
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	414.5



Desthiobiotin-TEG Azide
[26-6725-XX]

Click here for a list of other Affinity Ligand Modifications.
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.

Desthiobiotin-TEG Azide is a desthiobiotin attached to a 15-atom mixed polarity triethylene glycol spacer with an azide group at the end. 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 Desthiobiotin-TEG Azide 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). The spacer acts to minimize steric hindrance between the desthiobiotin moiety and the oligo.

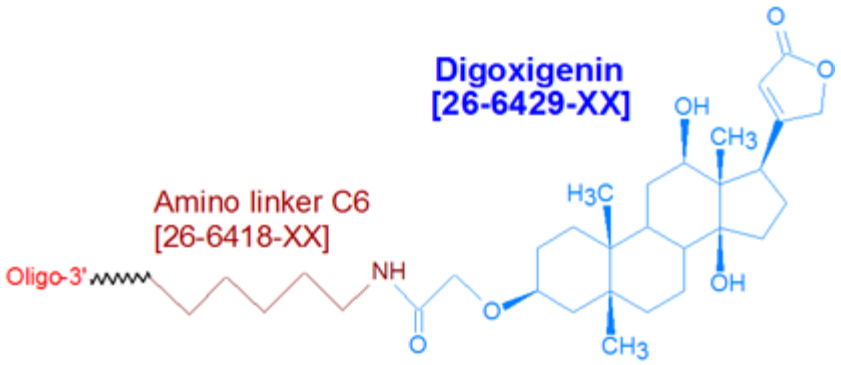
Like biotin, desthiobiotin binds to streptavidin, but its binding affinity is considerably less (2x10E-9 M) than that of biotin (4.0x10E-14 M) (3). Consequently, oligonucleotides labeled with desthiobiotin can be easily displaced from streptavidin by biotin, thereby making recovery of the labeled oligo (for example, in affinity purification protocols) from a streptavidin-coated support a relatively simple process (4). Desthiobiotin-labeled oligos can also be conveniently eluted from streptavidin-coated supports by incubation in distilled water at 95C for 10 minutes (5).

Gene Link recommends substitution of desthiobiotin for biotin for those cases where reversible capture of oligonucleotides is desirable. **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. Green, N.M. Spectrophotometric determination of avidin and biotin. *Methods Enzymol.* (1970), **18A**: 418-424.
4. Hirsch, J.D., Eslamizar, L., Filanoski, B.J., Malekzadeh, N., Haugland, R.P., Beechem, J.M., Haugland, R.P. Easily reversible desthiobiotin binding to streptavidin, avidin, and other biotin-binding proteins: uses for protein labeling, detection, and isolation. *Anal. Biochem.* (2002), **308**: 343-357.
5. van Doom, R., Slawiak, M., Szemes, M., Dullemans, A.M., Bonants, P., Kowalchuk, G.A., Schoen, C.D. Robust Definition and Identification of Multiple Oomycetes and Fungi in Environmental Samples by Using a Novel Cleavable Padlock Probe-Based Ligation Detection Assay.*Appl. Environ. Microbiol.* (2009), **75**: 4185-4193.

Digoxigenin NHS

Category	Affinity Ligands
Modification Code	Dig-N
Reference Catalog Number	26-6429
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	561.3



This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol * The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. * Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites.

Click here for a list of conjugation chemistry modifications. **Maleimide Ligand conjugation** requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.

Click here for a list of conjugation chemistry modifications.

Click Chemistry Ligand conjugation requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide,
BCN:Tetrazine and TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

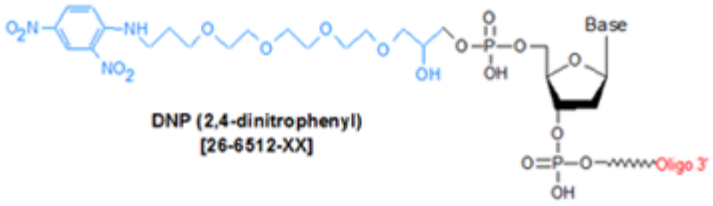
Digoxigenin (as Digoxigenin-3-O-methylcarbonyl-epsilon-aminocaproic acid NHS ester) is a member of the steroid family found in Digitalis plants (1). It is a hapten, that is, a small molecule having high immunogenicity. Because antibodies raised against haptens have considerably higher affinities for them than other antibodies do for their targets makes haptens particularly desirable as affinity tags for oligonucleotides (2).

Digoxigenin ('Dig') is commonly used to label oligonucleotides probes for use in hybridization applications, for example, in situ hybridization (3), Northern and Southern blotting. After hybridization to their targets, these Dig-labeled probes are detected with anti-Dig antibodies that are labeled with dyes (for primary detection) or enzymes (for secondary detection using a fluorogenic, chemiluminogenic, or colorimetric substrate specific for the enzyme). To maximize signal, Gene Link recommends modifying the oligonucleotide probe with three or more Dig molecules, spaced about 10 bases apart. Note that since digoxigenin is in the form of an NHS ester, an active primary amino group (such as Amino Linker C6) must first be incorporated into the oligonucleotide, to allow for subsequent conjugation to the digoxigenin NHS ester. **References**

1. Polya, G. *Biochemical targets of plant bioactive compounds.* New York: CRC Press, 2003. p 847.
2. Shreder, K. Synthetic Haptens as Probes of Antibody Response and Immunorecognition. *Methods (Academic Press)* (2000), **20**: 372-379.
3. Hauptmann, G., Gerster, T.. Two-color whole-mount in situ hybridixation to vertebrate and Drosophila embryos.*Trends Genet.* (1994), **10**: 266.

DNP TEG (2, 4-dinitrophenyl)

Category	Affinity Ligands
Modification Code	DNP-TEG
Reference Catalog Number	26-6512
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	509.41



DNP (2,4-dinitrophenyl)
[26-6512-XX]

DNP (2,4-dinitrophenyl) is classified as a hapten for molecular biology purposes, that is, a small molecule having high immunogenicity. Because antibodies raised against haptens have considerably higher affinities for them than other antibodies do for their targets makes haptens particularly desirable as affinity tags for oligonucleotides (1).

DNP attached to a triethylene glycol (TEG) spacer arm is commonly used to label oligonucleotides probes for use in hybridization applications, for example, in situ hybridization, Northern and Southern blotting (2). After hybridization to their targets, these DNP-labeled probes are detected with anti-DNP antibodies that are labeled with dyes (for primary detection) or enzymes (for secondary detection using a fluorogenic, chemiluminogenic, or colorimetric (3) substrate specific for the enzyme). To maximize signal obtained with such probes, Gene Link recommends modifying the oligonucleotide probe with three DNP molecules, either grouped at the 5'-end or spaced about 10 bases apart (2).

In addition to the above straightforward anti-DNP antibody-based detection systems, oligo probes labeled with both a fluorescent dye and DNP also been used for highly-sensitive direct detection of antigens (at femtoMolar levels) in a rolling circle amplification (RCA)-based assay system (4). **References**

1. Shreder, K. Synthetic Haptens as Probes of Antibody Response and Immunorecognition. *Methods (Academic Press)* (2000), **20**: 372-379.
2. Grzybowski, J., Will, D.W., Randall, R.E., Smith, C.A., Brown, T.. Synthesis and antibody-mediated detection of oligonucleotides containing multiple 2,4-dinitrophenyl reporter groups. *Nucleic Acids Res.* (1993), **21**: 1705-1712.
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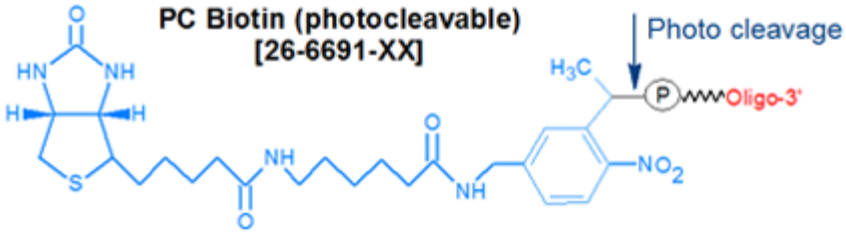
PC Biotin (photocleavable)

Category	Photo Cleavable
Modification Code	PCBio
Reference Catalog Number	26-6691
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	597.62



Click here for a list of other Affinity Ligand Modifications.

PC Biotin (photocleavable) is a non-nucleosidic molety that can be used to incorporate a UV photo-cleavable biotin molecule onto the 5'- end of an oligonucleotide. The biotin is separated from the 5'- end nucleotide base by the photo-cleavable group and a long-chain alkyl spacer arm to minimize steric interaction between the biotin and the oligo (1). The photo-cleavable group, located on the 5'- phosphate, can be selectively cleaved by illumination with UV light quantitatively in less than 4 minutes, thereby releasing the biotin to produce a 5'- phosphorylated oligo (1). PC Biotin thus allows researchers a facile method for streptavidin-mediated affinty capture and release of biotinylated oligos or PCR products in purification or diagnostic applications. In the case of a PCR product, retainment of the 5'- phosphate also makes it suitable for cloning.

Besides the above applications, PC Biotin-modified oligos could be used to isolate different kinds of DNA or RNA macromolecular complexes, such as nucleosomes (2) and chromatin (3).

PC Biotin could also be used to create 'caged' oligonucleotides, that is, oligonucleotides whose activity is suppressed until released by an external factor (such as UV light). Caging oligonucleotides (for example, tethering anti-sense or siRNA, via PC Biotin, to a molecule that suppressed its activity) would provide new possibilities for controlling biological mechanisms (such as gene expression) in space and time (4).

Cleavage Protocol
Cleavage occurs by irradiation with near-UV light (300-350 nm, complete cleavage occurs within 5 minutes. Try using a Black Ray XX-15 UV lamp (Ultraviolet Products Inc., San Gabriel, CA) at a distance of 15 cm (emission peak 365 nm, 300 nm cut-off, 1.1 mW intensity at~31 cm).

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