



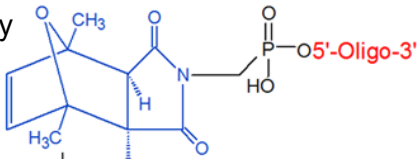

Product Specifications

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.

Maleimide (5')

Category	Conjugation Chemistry	
Modification Code	Mal	
Reference Catalog Number	26-6574	
5 Prime	Y	
3 Prime	N	
Internal	N	
Molecular Weight(mw)	203.09	

MW Note: The molecular weight of 203.09 for maleimide is for the completely deprotected form after retro-Diels Alder reaction. Prior to retro-Diels Alder reaction the MW is 299.22. Gene Link provides maleimide modified oligos that requires retro-Diels Alder reaction by the user. See protocol below.

Maleimide Modifier (5') can be used to directly incorporate an active maleimide moiety onto the 5'-end of an oligonucleotide. The maleimide is separated from the 5'-end nucleotide base by a 2-carbon spacer arm to reduce steric interaction between it and the oligo. Maleimide-labeled oligos are typically used to form conjugates with thiol-labeled moieties, or Diels-Alder cycloaddition products.

As the maleimide moiety itself is not stable over the long-term, Gene Link provides lyophilized maleimido-oligos with all base protecting groups removed and as a maleimide-2,5-dimethylfuran cycloadduct. The cycloadduct serves to protect the maleimide from degradation. The customer then converts the cycloadduct to the active maleimide via a retro-Diels-Alder reaction using toluene and heat. The conversion protocol is provided with the modified oligo. If required the protected oligo should be aliquoted in smaller portions and stored dry. The retro-Diels-Alder reaction should be performed immediately prior to conjugation.

Deprotection by heating in Toluene

Gene Link provides the oligo in a dried lyophilized form. The maleimide is provided in a protected state and deprotection by Retro Diels-Alder reactions is required before use by the end user for conjugation.

The Retro Diels-Alder reactions involves dehydration by co-evaporation with anhydrous acetonitrile and anhydrous toluene. The Retro Diels-Alder reaction requires anhydrous conditions and any significant level of moisture can cause incomplete deprotection, hydrolysis, and/or addition of water to the maleimide. The evaporation of the toluene leaves a white residue ready for conjugation. A detailed procedure is shipped with the product. An abbreviated procedure is described below.

1. Suspend lyophilized oligo in 1 mL anhydrous Acetonitrile. It will be a suspension as oligo will NOT dissolve.
2. Evaporate using a speedvac.
3. Resuspend oligo in 1.5 mL Toluene.
4. Incubate for 4 hrs at 90° C.
5. Cool to room temperature.
6. Evaporate toluene using a speedvac.

7. The oligonucleotide is now ready for conjugation.
Detailed deprotection Retro Diels-Alder reactions protocol Retro Diels-Alder Reaction protocol.