



## 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.

### Thiol SS-C6

Category Conjugation Chemistry

Modification Code SS-C6

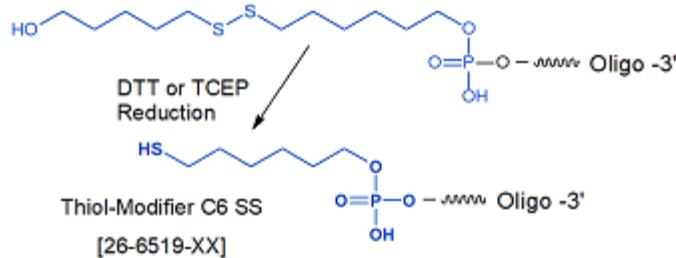
Reference Catalog Number 26-6419

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 196.2



Note 1: Thiol-SS-C6 when incorporated internally will cleave the oligo upon reduction at the S-S site.

Note 2: The molecular weight added to the oligo mw calculation for thiol modification is 196.2 for the reduced form. The oligo is supplied in the unreduced form with disulfide thiol mw of 328.4. The oligo with the unreduced form of disulfide is for the end user to perform the reduction before use.

Prior to use, reduce any disulfide formation using 100 mM TCEP or DTT for 30 minutes at room temperature. TCEP 0.5M solutions are available from Gene Link, Catalog Number: 40-5116-10. TCEP use is recommended for reduction as conjugation efficiency are 2-3% higher if TCEP is used. TCEP 0.5M solution

#### Thiol Reduction Protocol

Thiol-SS-C6 is a disulfide-containing modifier designed to functionalize an oligonucleotide with a reactive thiol (sulphydryl) group at the 5'- or 3'-end, or an internal disulfide linkage. This modification is incorporated into the oligonucleotide as a disulfide during oligonucleotide synthesis, in order to protect the thiol group from undesired side reactions. After synthesis, Gene Link normally supplies the oligo to the customer in the **oxidized (disulfide)** form. The disulfide bond can then be reduced with TCEP or dithiothreitol (DTT) to generate the fully active thiolated oligo by the customer in his/her own laboratory. The resulting thiol group is separated from the oligo by a six-carbon spacer arm, to reduce steric interactions with the end of the oligo. Note that if this modification is incorporated internally, it can effectively serve as a C12 spacer between two oligos, with a disulfide linkage in the middle. **Reduction of the disulfide bond will generate two separate 3'- and 5'-thiolated oligos, respectively.**

Thiolated oligonucleotides can be labeled with thiol-reactive dye / happen iodoacetamides or maleimides (for example, lucifer yellow iodoacetamide or fluorescein maleimide) for use as hybridization or PCR-based detection probes (1). They also can be conjugated to enzymes (for example, alkaline phosphatase or horseradish peroxidase), through bifunctional linkers (2).

Finally, thiolated oligos can be attached to glass slides or gold surfaces for use in various microarray or nanoelectronic applications (3,4). However, because oligos labeled with only one thiol slowly dissociate from a gold surface at the temperatures (60C to 90C) and high salt concentrations commonly used to denature DNA duplexes (5), Gene Link recommends that researchers who plan to use such conditions to repeatedly strip and re-probe oligo arrays based on thiol-gold surface conjugation modify the oligos with DTPA, which incorporates two thiol groups into the oligo, thereby allowing for a more stable attachment to gold. For further information on DTPA, please see its technical sheet.

## References

1. Connolly, B.A., Rider, P. Chemical synthesis of oligonucleotides containing a free sulphydryl group and subsequent attachment of thiol specific probes. *Nucleic Acids Res.* (1985), **13**: 4485-4502.
2. Ghosh, S.S, Kao, P.M., McCue, A.W., Chappelle, H.L. Use of maleimide-thiol coupling chemistry for efficient syntheses of oligonucleotide-enzyme conjugate hybridization probes. *Bioconjugate Chem.* (1990), **1**: 71-76.
3. Roger, Y-H., Jiang-Baucom, P., Huang, Z-J., Bogdanov, V., Anderson, S., Boyce-Jacino, M.T. Immobilization of oligonucleotides onto a glass support via disulfide bonds: A method for preparation of DNA microarrays. *Anal. Biochem.* (1999), **266**: 23-30.
4. Ackerson, C.J., Sykes, M.T., Kornberg, R.D. Defined DNA/nanoparticle conjugates. *Proc. Natl. Acad. Sci. USA* (2005), **102**: 13383-13385.
5. Li, Z., Jin, R., Mirkin, C.A., Letsinger, R.L. Multiple thiol-anchor capped DNA-gold nanoparticle conjugates *Nucleic Acids Res.* (2002), **30**: 1558-1562.