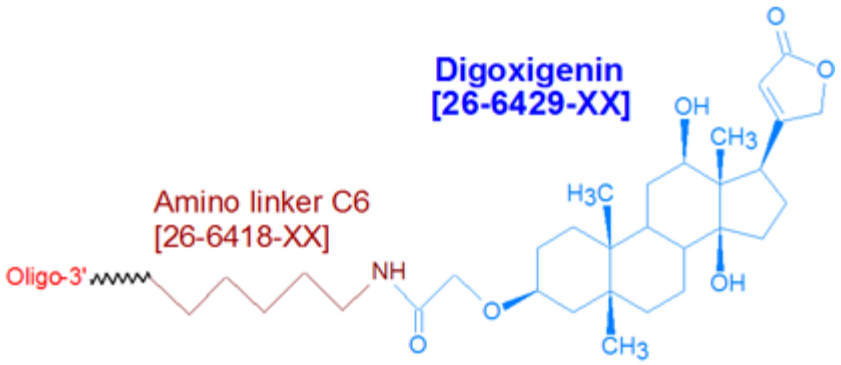
Digoxigenin NHS

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** 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.

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.