### 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.

#### NHS-Carboxy-dT

<table>
<thead>
<tr>
<th>Category</th>
<th>End Modifiers</th>
<th>Modification Code</th>
<th>Reference Catalog Number</th>
<th>Molecular Weight(mw)</th>
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<tr>
<td></td>
<td></td>
<td>NHS-CO dT</td>
<td>26-6453</td>
<td>475.3</td>
</tr>
<tr>
<td>5 Prime</td>
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<td></td>
<td></td>
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<tr>
<td>3 Prime</td>
<td>Y</td>
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<tr>
<td>Internal</td>
<td>Y</td>
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Depending on your specific application, Gene Link can provide Carboxy dT modified oligos either with the NHS-protected with oligo bound to CPG solid support or can conjugate the NHS-Oligo to user specified amino-ligand.


The carboxy-dT is hydrolyzed during deprotection and can be coupled directly to a molecule containing a primary amino group by a standard peptide coupling or via the intermediate N-hydroxysuccinimide (NHS) ester. Amino-Modifier dA, Amino-Modifier dC, Amino-Modifier dG and both Amino-Modifier dT products can be added in place of a dA, dC, dG and dT residue, respectively, during oligonucleotide synthesis. Corresponding Amino-Modifier supports can replace their respective deoxynucleoside supports. After deprotection, the primary amine on the C6 analogues is separated from the oligonucleotide by a spacer arm with a total of 7 -10 atoms and can be labelled or attached to an enzyme. The C2 analogue is more suitable for the attachment of molecules designed to react with the oligonucleotide.

#### Standard Conjugation Protocol Using Solid Phase NHS-Oligo-CPG

Ensure that the amino-ligand to be conjugated to the NHS-Oligo bound to CPG is stable to exposure to ammonium hydroxide solution (30%) for ~24 hrs. The ammonium hydroxide treatment is required to cleave the oligo from CPG bound oligo after the NHS conjugation is performed.

1. NHS-oligo labels almost quantitatively rapidly at room temperature. Keep reaction for 15 minutes or up to 3 hrs.
2. Split NHS-Oligo-CPG into two 2mL Screw Cap tubes. It is prudent to test one and then the second tube.
3. Should have at least 4 mg of amino ligand for 200 nmol scale synthesis oligo on CPG.
4. Prepare 200 uL of DMSO or DMF containing 1% diisopropylethylamine [CAS: 7087-68-5] or 10% Triethylamine [CAS: 121-44-8].
5. Dissolve ~4 mg amino-Ligand in 200 uL DMSO or DMF, containing 1% diisopropylethylamine [CAS: 7087-68-5] or 10% Triethylamine [CAS: 121-44-8].
6. Allow to react at room temperature for 15 to 30 minutes or longer. Vortexing intermittently.
7. Centrifuge at 3K rpm for 30 seconds.
8. Aspirate with a pipet unreacted ligand and save for future use.
9. Wash Oligo-CPG with 0.5 mL acetonitrile or DMF.
10. Repeat step 9 three times.
11. Dry CPG at room temperature. This is now labelled with the amino-ligand to the NHS group on the oligo bound to CPG.
12. Deprotect oligo and cleave from CPG by adding 1 mL cold 30% ammonium hydroxide (stored in a freezer) and keep 18-24 hrs. at room temperature. It is essential to incubate in screw cap tubes with cap closed securely to prevent ammonia leakage. Vortex occasionally.
13. Open caps of the tube inside a hood and leave it for nearly 30 minutes for ammonia to evaporate.
14. Gently aspirate the ammonia solution using a pipet. Transfer to a fresh 2 mL screw cap tube.
15. Add 0.5 mL sterile water to the CPG, vortex gently and aspirate the solution using a pipet. Pool with the previous aspirated solution.
16. Dry the solution using a speedvac.
17. Dissolve pellet in 0.4mL sterile water. Add 50 uL of 3 M Sodium Acetate pH 5.2 and 1 mL ethanol. Incubate at -20 C for 20 minutes.
18. Centrifuge at 12K rpm for 5 minute. Decant ethanol carefully so as not to disturb the pellet.
19. Add 0.5 mL 70% ethanol, vortex and centrifuge at 12K rpm for 5 minute.
20. Dry pellet at room temperature.
21. The pellet contains the oligo conjugated to your amino ligand. Process for purification if desired by HPLC or 7M urea-polyacrylamide gel.