NHS-Carboxy-dT

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.

Gene Link provides custom oligo modified with NHS-carboxy C10 and NHS-Carboxy dT bound to CPG (controlled pore glass). The oligo is in a protected form and has to be deprotected and cleaved from the CPG after completion of the amino ligand conjugation to NHS.

The protocol given below has been tested with amino labeled dyes and other amino ligands soluble in DMSO and DMF.

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.