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Modify and label oligonucleotide 5´ phosphate groups

TR0030.5

Introduction

The 5´ phosphate group of oligonucleotides, DNA and RNA can be conjugated to primary amine-containing molecules using the carbodiimide crosslinker EDC (Part No. 22980) and imidazole. The example protocol presented in this Tech Tip describes the amine-modification of an oligonucleotide with an excess of ethylenediamine, as illustrated in Figure 1. Depending on the amine-containing molecules used, the crosslinking strategy can be adapted in a number of ways to directly or indirectly modify, label or conjugate an oligonucleotide (Table 1).

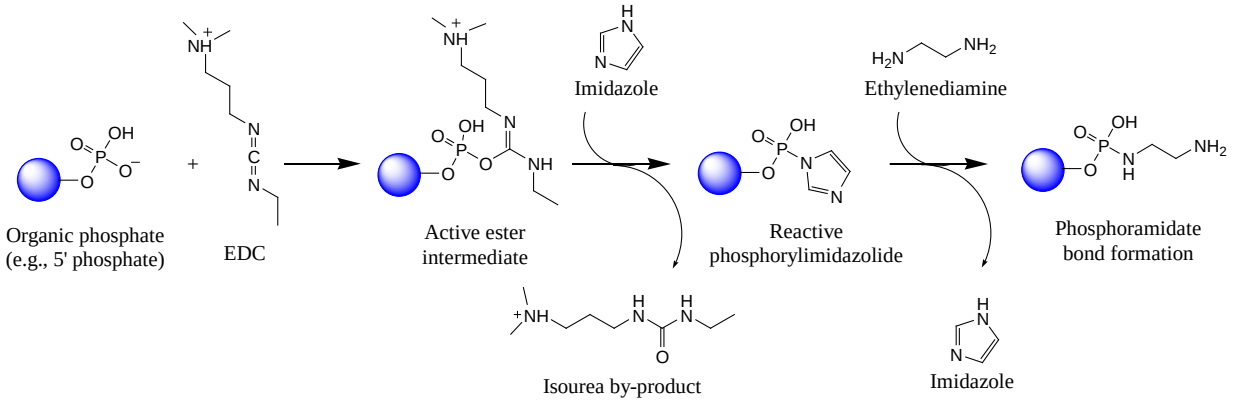

Figure 1. Amine-modification of oligonucleotide using ethylenediamine, EDC and imidazole.

Table 1. Possible applications for EDC/imidazole-mediated modification of oligonucleotides.

Conjugation Goal	Reagent (Thermo Scientific Part No.) and Strategy
Biotin-label the oligonucleotide	Use Biotin Hydrazide (21339) or Biotin-LC-Hydrazide (21340) instead of ethylenediamine in the default reaction.
Create a sulfhydryl-reactive oligonucleotide	Use EMCH (22106), KMUH (22111) or MPBH (22305) instead of ethylenediame in the default reaction. Use PDPH (22301) instead of ethylenediamine to obtain a sulfhydryl crosslink that is reversible. This strategy is useful for preparing conjugates with sulfhydryl-containing proteins and other molecules.
Create a sulfhydryl group on oligonucleotide	Use cystamine (H_2N-CH_2-CH_2-S-S-CH_2-CH_2-NH_2) instead of ethylenediamine in the default reaction, and then reduce the disulfide bond with DTT or similar reagent. This strategy is useful for preparing oligo-enzyme conjugates (by reaction to maleimide-activated enzymes, such as Part No. 31485) for assays and blotting procedures.
Immobilize oligonucleotide to a beaded affinity support	Use UltraLink Hydrazide (53149) instead of ethylenediamine in the default reaction. This strategy is useful for preparing supports for affinity purification of binding partners.
Fluorophore-label the oligonucleotide	Amine-modify the oligo with ethylenediamine using the default procedure, then react the amino group with the desired amine-reactive fluorophore (e.g., Fluorescein, 46410 or 46425; DyLight™ 550, 62262; or DyLight 650, 62265)

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Materials Required

- EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride), Part No. 22980
- Ethylenediamine (Part No. 23031) or other amine- or hydrazide-containing molecule
- 0.1 M Imidazole, pH 6
- Reaction Buffer, such as phosphate buffered saline (PBS) with EDTA: 10 mM sodium phosphate, 0.15M NaCl, 10mM EDTA, pH 7.2. Avoid using PBS with > 10mM phosphate, which will interfere with the intended reaction. Other amine-free and carboxylate-free buffers may be substituted, but avoid Tris, which contains a primary amine that will quench the reaction.
- 7.5-15nmol (~60-120µg) oligonucleotide or double-stranded DNA or RNA dissolved in ~10µL Reaction Buffer.

Procedure for Modifying 5´ Phosphate Groups

1. Dissolve ethylenediamine (or alternative) to a final concentration of 0.25M in 10µL of 0.1M imidazole.
2. Weigh 1.25mg (6.52µmol) of EDC into a microcentrifuge tube.
3. Add 7.5µL of the prepared oligonucleotide to the tube containing the EDC and immediately add 5µL of the ethylenediamine/imidazole solution.
4. Vortex tube until contents are completely dissolved, and then briefly centrifuge the tube to gather contents.
5. Add an additional 20µL of 0.1M imidazole, pH 6.
6. Incubate reaction at one of the following conditions:

Temperature	Time
50°C	30 minutes to 2 hours
37°C	1 hour to overnight
Room Temperature	2 hours to overnight

7. Remove non-reacted EDC and its by-products and imidazole by dialysis (e.g., Slide-A-Lyzer® MINI Dialysis Units) or spin desalting column (Zeba™ Spin Desalting Column) using 10mM sodium phosphate, 0.15M NaCl, 10mM EDTA, pH 7.2, or other suitable buffer.

   Note: The amine-modified oligonucleotide may be stored frozen for up to one year or used immediately for other conjugation reactions. If heterobifunctional hydrazide compounds were used, purify and store the conjugate in a manner suitable for stability of the second reactive group.

Additional Information

The following reference (Part No. 20036) provides further discussion of EDC-mediated oligonucleotide modification and other oligonucleotide crosslinking techniques, as well as citations of original literature:

Hermanson, G.T. (2008). Bioconjugate Techniques. 2nd Edition. Academic Press, San Diego.