

Modifications for Antisense Applications

Description

The goal of antisense applications is to shut down activity of a defined gene by blocking transcription or translation within cells.

Oligonucleotides for antisense assays must be nuclease resistant against cellular nucleases, must be able to cross cellular membranes and must inherit both high binding affinity and specificity for the target sequence. In many cases, they also must have the ability to induce RNase H cleavage.

Advantages

- enabling to shut down defined genes
- high stability of oligonucleotides

Applications

- basic molecular biology research to elucidate gene functions and cellular pathways (usually by blocking gene expression)
- substrates for molecular biochemistry (enzyme screening, characterization, kinetics)
- therapeutic research

Phosphothioate Oligonucleotides (PTOs)

PTOs contain one sulfur atom in place of an oxygen atom in the internucleotide linkage of DNA or RNA. They are extremely useful as **antisense** molecules inhibiting gene expression, because they are more resistant to nuclease degradation than natural DNA or RNA and still bind to complementary nucleic acid

sequences. The more PTO linkages present in an oligonucleotide, the higher its stability. Phosphothioate oligonucleotides can be ordered as full PTOs (whole oligonucleotide containing PTO linkages), as “thio-cap” oligonucleotides, (with only a few PTO linkages at the respective 3'- and/or 5'- ends) or as chimers with several stretches of PTOs interspersed in the oligonucleotide.

Full PTOs are more stable than “thio-cap” oligonucleotides, but due to their extreme stability and their hydrophobic character they can also have toxic effects on living cells. If cell toxicity is a problem in your assay, we recommend that you use “thio-cap” oligonucleotides instead. “Thio-caps” can even further stabilize 2'-O-Me-RNA.

The phosphorous atoms within PTO backbones are optically active. If you wish, Thermo Electron's biopolymer specialists can even separate and purify the different stereoisomers of molecules with **one** PTO linkage.

The sulfur atoms within PTO backbones are chemically reactive (but less reactive than “normal” thiol groups). Thus, they offer another possibility of modifying your oligonucleotide even further.

In general, modifications are stable against enzymatic digestion, but in some cases, PTO linkage of modifications to oligonucleotides can stabilize them.

Please indicate the desired linkage type when ordering.

PTOs are one of the least expensive ways to stabilize oligonucleotides.

If you are in need of further information, please refer to our special Tech Info on PTOs ID: TI-OL05-1104.

2'- O-Methyl-RNA-Oligonucleotides

These molecules carry a methyl group at the 2'-OH residue of the ribose molecule. 2'-O-Me-RNAs show the same behavior as DNA, but are protected against nuclease degradation.

Such oligonucleotides form more stable hybrids with complementary RNA strands compared to DNA or RNA. They are extremely efficient in blocking RNA functions or, if modified with a specific label (p.e. biotin), they can be used in affinity purification of specific RNAs. 2'-O-Me-RNAs can be combined with phosphothioate oligonucleotides (PTOs) for further stabilization.

If you are in need of further information, please refer to our special Tech Info on 2'-O-Me-RNAs ID: TI-OL02-1104.

Methyl-Phosphonate Oligonucleotides

These molecules carry a methyl group at their internucleotidic linkage. Methyl-phosphonates are extremely stable and do no longer behave like normal nucleic acids. Methyl-phosphonate linkages can be used to protect the 3'- and/or 5'-ends of DNA in **antisense** experiments. Due to their low solubility and product yield, a maximum of 3 Methyl-Phosphonate linkages on either end of your DNA should not be exceeded.

If you are in need of further information, please refer to our special Tech Info on Methyl-Phosphonate Oligonucleotides ID: TI-OL04-1104.

Inverted End (3'-3'-linkage):

A very efficient method to stabilize oligonucleotides against enzymatic degradation is to couple the 3'-terminal base via a 3'-3' linkage. As this kind of linkage is not recognized and/or digested by most enzymes, these oligonucleotides are more stable in living cells than normal DNAs.

If you are in need of further information, please refer to our special Tech Info on Inverted End Modifications ID: TI-OL03-1104.

siRNA

siRNAs (small interfering RNAs) are short double-stranded RNA molecules that induce sequence specific posttranscriptional gene silencing - a mechanism called RNA interference (RNAi).

siRNA molecules are typically made of two hybridized strands of 18-20 RNA bases, each with 2 DNA bases that overhang at the 3' terminus.

If you are in need of further information, please refer to our special Tech Info on siRNA ID: TI-OL06-1104.

Product offering

Easily order all products via the web:

www.thermo.com/siRNA

or via email:

sales.oligos@thermo.com

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