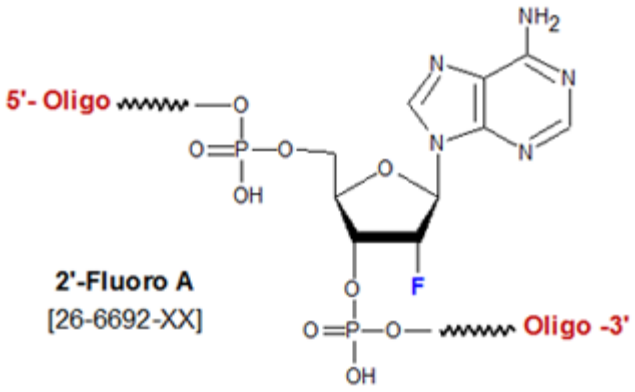
Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

2'-F-A

Category	Nuclease Resistance
Modification Code	fA
Reference Catalog Number	26-6692
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	331.2



Antisense Oligos (ODN) & siRNA Oligo Modifications
Click here for more information on antisense modifications, design & applications.

2'-Fluoro-deoxyadenosine (2'-F-A) is classified as a 2'-Fluoro RNA monomer. The substitution of a highly electronegative fluorine atom for the 2'-OH group of the ribose results in the ring of a 2'-F-ribonucleoside having a C3'-endo (i.e., RNA-type) conformation. Consequently, an RNA oligonucleotide containing a 2'-F RNA monomer adopts the more thermodynamically stable A-form helix on hybridization to a target (1).

2'-F RNA modifications have been shown to be particularly useful in the following oligo-based applications:

(a) Anti-sense oligos & Nuclease Resistance: When 2'-F RNA is incorporated into an anti-sense DNA oligo (resulting in a 2'-F RNA/DNA chimeric), the Tm of its duplex with RNA **increases** relative to that formed by an unmodified anti-sense DNA oligo **by 1.8°C per 2'-F RNA residue added** (1). The DNA/RNA duplex formed between a chimeric 2'-F-RNA/DNA anti-sense oligo and its RNA target also is a substrate for RNase H. With respect to nuclease resistance, while 2'-F RNA nucleotides do provide some nuclease resistance when incorporated into DNA, phosphorothiolation of the 2'-F RNA phosphodiester linkages is recommended, because it strongly enhances such resistance. This effect becomes particularly important if the 2'-F RNA nucleotide is to be incorporated at one or more of the first three positions of the 5'- or 3'-ends of the oligo.
Modifications Increasing Duplex Stability and Nuclease Resistance

Modification

Duplex Stability [Tm Increase]

Nuclease Resistance Locked Analog Bases Increased [2- 4C per substitution] Increased 2-Amino-dA Increased [3.0C per substitution] Similar to DNA C-5 propynyl-C Increased [2.8C per substitution] Increased C-5 propynyl-U Increased [1.

7C per substitution] Increased 2'-Fluoro Increased [1.8C per substitution] Increased 5-Methyl-dC Increased [1.3C per substitution] Similar to DNA 2'-O Methyl Increased Increased Phosphorothioate Slightly decreased Increased Click here for complete list of duplex stability modifications

(b) Aptamers: The addition of 2'-F pyrimidines (C, U) into RNA aptamers has been shown to provide them with both considerably increased nuclease resistance and equal or higher binding affinity for their ligands, compared to the corresponding unmodified aptamers (2,3). The incorporation of 2'-F RNA nucleotides into RNA aptamers as an optimization strategy is becoming an increasingly common practice.

(c) siRNA & Nuclease Resistance: siRNA synthesized with 2'-F pyrimidines have been shown to have greatly increased stability in plasma compared to 2'-OH siRNA (4,5). In one study, levels of inhibition for 2'-F siRNA and 2'-OH siRNA, in cell culture and *in vivo* using BALB/c mice transfected with pGL3 luciferase, were similar over time (4). In another study, siRNA fully substituted with both 2'-F RNA and 2'-O-Methyl RNA nucleotides displayed not only enhanced stability in plasma, but also >500-fold increase in capability to down-regulate gene expression compared with the corresponding unmodified siRNA (5).

(d) LNA Alternative: The increased thermal stability and nuclease resistance provided by 2'-F RNA residues make them attractive as lower-cost substitutes in any application involving LNA-modified oligos.

ASO's and siRNA Modifications.
Click this link to view ASO's and siRNA Modifications.

ASO's and siRNA Delivery. The development of effective delivery systems for antisense oligonucleotides is essential for their clinical therapeutic application. The most common delivery system involves a relatively hydrophobic molecule that can cross the lipid membrane. Cholesterol TEG, alpha-Tocopherol TEG (a natural isomer of vitamin E), stearyl and GalNAc modifications have been shown to effective for delivery of ASO's and siRNA in addition to cell penetrating peptides.
Click this link to view these modifications.

REFERENCES:

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3. Goringer, H.U.; Adler, Annette; Forster, Nicole; Homann, Matthias. Post-SELEX Chemical Optimization of a Trypanosome-Specific RNA Aptamer. *Combinatorial Chemistry & High Throughput Screening* (2008), **11**: 16-23.
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