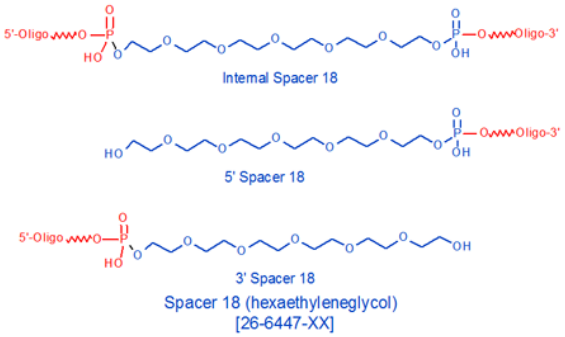
Oligo Modifications

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

Spacer 18

Category	Spacers
Modification Code	Sp18
Reference Catalog Number	26-6447
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	344.3



Spacer 18 also known as PEG6 is a hexaethylene glycol chain that is 18 atoms long (12 carbons + 6 oxygen's), and is used to incorporate a long spacer arm into an oligonucleotide. Spacer 18 can be incorporated in consecutive additions whenever a longer spacer is required. Spacer 18 had been used to form bold folds and hairpin loops in oligonucleotides (1,2), and for solid-phase immobilization of hybridization probes (3). Spacer 18 has also been used to modify random primers used in whole genome amplification (WGA)-based applications, as a way to eliminate self-priming events that form spurious DNA products (that is, false-positive amplification) in the PCR reactions (4).
Gene Link offers spacers of various length, examples C2, C3, C6, C12 and Spacer 9 and 18. These can be inserted multiple times to yield a total much longer spacer length. **References**

1. Salunkhe, M., Wu, T.F., Letsinger, R.L. Control of folding and binding of oligonucleotides by use of non-nucleotide linker. *J. Am. Chem. Soc.* (1992), **114**: 8768-8772.
2. Durand, M., Chevrie, K., Chassignol, M., Thuong, N.T., Maurizot, J. Circular dichroism studies of an oligodeoxyribonucleotide containing a hairpin loop made of a hexaethylene glycol chain: conformation and stability.*Nucleic Acids Res.* (1990), **18**: 6353-6359.
3. Zhang, Y., Coyne, M.Y., Will, S.G., Levenson, C.H., Kawasaki, E.S. Single-base mutational analysis of cancer and genetic diseases using membrane bound modified oligonucleotides. *Nucleic Acids Res.* (1991), **19**: 3929-3933.
4. Brukner, I., Paquin, B., Belouchi, M., Labuda, D., Krajinovic, M. Self-priming arrest by modified random oligonucleotides facilitates the quality control of whole genome amplification.*Anal. Biochem.* (2005), **339**: 345-347.

Oligonucleotide PEGylation : Spacers vs. PEGylation Gene Link offers short PEG3 and PEG6 as direct coupling using automated chemistry. The PEG3 is termed as Spacer 9 and PEG6 as spacer 18. These are also used to introduce space between adjacent sequence and modifications. These can be inserted multiple times to increase the PEG units.
Larger 2, 5, 10 and 20 kDa PEGylation of oligonucleotides is inserted at any site of an oligonucleotide using a post synthesis amino group on the oligo with PEG-NHS.
PEGylation is the covalent attachment of polyethylene glycol (PEG) to oligonucleotides such as DNA, RNA, antisense, siRNA and aptamers.

It improves pharmacokinetics, reduces renal clearance, increases nuclease stability, and decreases immunogenicity. (1) The way PEG shields its conjugated payload offers new challenges and opportunities for oligonucleotide PEGylation. Other than aptamers, the target of most oligonucleotides is a complementary sequence.
Comparison of PEGylation Size & Biological Outcome

PEG Size (Ethylene Glycol Units)

Hydrodynamic Effect

Typical Outcome 2 kDa (~44) Minimal Slight stability increase 5 kDa (~114) Moderate Partial half-life improvement 10 kDa (~227) Strong Reduced renal clearance 20 kDa (~455) Very strong Long circulation 40 kDa (~910) Extreme Depot-like behavior Pharmacokinetics depend on hydrodynamic diameter, not molecular weight of the oligo. Unmodified 20-mer oligo ≈ ~7 kDa. Kidney filtration cutoff ≈ 40-60 kDa hydrodynamic equivalent Oligo PEG size controls circulation time versus tissue penetration. Optimal design balances exposure and activity
Messenger RNA (mRNA) delivery strategies are required to protect biologically fragile mRNA from ribonuclease (RNase) attacks to achieve efficient therapeutic protein expression. To tackle this issue, most mRNA delivery systems have used cationic components.
A cation-free delivery strategy by hybridization of PEGylated RNA oligonucleotides with mRNA. The PEG strands on the mRNA sterically and electrostatically shields the mRNA, improving mRNA nuclease stability 15-fold and the PEGylated mRNA induced nearly 20-fold higher efficiency of reporter protein expression than unhybridized mRNA in cultured cells (2).
PEGylation has been used to improve the biopharmaceutical properties of protein drugs since the 1990s, and over a dozen PEGylated pharmaceuticals are currently on the market (2). PEG creates a large hydration shell, which sterically blocks other biomacromolecules from penetrating through the polymer layer and binding with the interior substrate (3, 4). Binding requires displacing the PEG by the incoming molecule, generally making such binding less thermodynamically favorable. These properties usually result in weaker interactions between the receptor and the conjugated molecule, but increased drug solubility, prolonged blood circulation, and increased drug stability often offset by the reduced binding affinity. PEGylated oligonucleotides can be an exception to this generalization, with increased binding to a complementary sequence compared to unmodified ONs. The effect is attributed to macromolecular volume exclusion (6).

PEGylation References

1. Li WJ; Zhan P; De Clercq E; Lou HX; Liu XY Current drug research on PEGylation with small molecular agents. Prog. Polym. Sci 2013, 38, 421-444.
2. Yoshinaga, N; Naito, M; Tachihara, Y; Boonstra, E; Osada, K; Cabral, H and Uchida, S. PEGylation of mRNA by Hybridization of Complementary PEG-RNA Oligonucleotides Stabilizes mRNA without Using Cationic Materials. Pharmaceutics 2021, 13, 800.
3. Harris JM; Chess RB Effect of pegylation on pharmaceuticals. Nat. Rev. Drug Discov 2003, 2, 214-221. [PubMed: 12612647]
4. Harris JM; Martin NE; Modi M Pegylation. Clin. Pharmacokinet 2001, 40, 539-551. [PubMed: 11510630]
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6. Nakano S-I; Karimata H; Ohmichi T; Kawakami J; Sugimoto N The effect of molecular crowding with nucleotide length and cosolute structure on DNA duplex stability. J. Am. Chem. Soc 2004, 126, 14330-14331. [PubMed: 15521733]