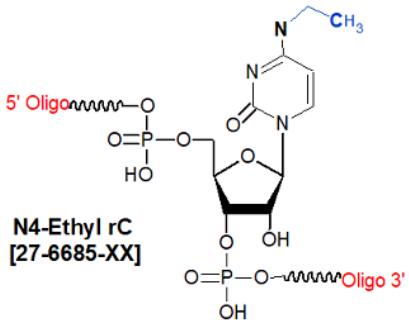
Oligo Modifications

N4-Ethyl rC [N4-Et-rC]

Category	Duplex Stability
Modification Code	N4-Et-rC
Reference Catalog Number	27-6685
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	333.4



N4-Ethyl-deoxycytidine (N4-Et-dC) is typically used to minimize the deleterious effect of large variations in GC content in target/probe sequences on the results produced by techniques involving simultaneous hybridization of many sequences, for example, DNA chip or reverse hybridization protocols (1). Due to the higher thermal stability of C:C base pairs, high-GC content sequences may contain mis-matches yet still stably hybridize to a probe or target (resulting in false positives), while low-GC content sequences may perfectly match to probe or target but the strands may dissociate upon washing (resulting in false negatives). This problem can be particularly acute in cases where the probes are short oligos (octamers, nonamers, etc.) A clever solution to this problem is to modify oligonucleotide probes to equalize (normalize) the thermal stability of G:C and A:T base pairs formed upon hybridization to the target, thereby making hybridization dependent only on oligo length and not on base composition. N4-Et-dC base pairs with dG, but the N4-Et-dC : dG base pair has a thermal stability similar to an A:T base pair instead of a C:G base pair. The dramatic effect on thermal stability was shown in two hybridization studies in which different sets of probes having GC content ranging from 0% to 100% were hybridized to their respective natural targets, and the Tm of the duplexes measured. For these unmodified probes, the Tm range was 39degC and 52degC, respectively. When N4-Et-dC was substituted for dC in these probes, the Tm range of the duplexes was only 7degC and 16degC, respectively (2,3).

N4-Et-dC-modified oligos have also been used in structure-function studies to better understand how CpG-containing oligos stimulate the innate immune system, and which structural elements in cytosine and guanine bases are required for recognition of, and interaction with, protein/receptor factors responsible for immunostimulation (4). **References**

1. Saiki, R.K, Walsh, P.S., Levenson, C.H., Erlich, H.A. Genetic analysis of amplified DNA with immobilized sequence-specific oligonucleotide probes. *Proc. Natl Acad. Sci. USA* (1989), **86**: 6230-6234.
2. Nguyen, H-K, Auffray, P., Asseline, U., Dupret, D., Thuong, N.T. Modification of DNA duplexes to smooth their thermal stability independently of their base content for DNA sequencing by hybridization. *Nucleic Acids Res.* (1997), **25**: 3059-3065.
3. Nguyen, H-K.

, Bonfils, E., Auffay, P., Costaglioli, P., et al. The stability of duplexes involving AT and/or G4EtC base pairs is not dependent on their AT/G4EtC ratio content. Implication for DNA sequencing by hybridization. *Nucleic Acids Res.* (1998), **26**: 4249-4258.
4. Kandimalla, E.R., Yu, D., Zhao, Q., Agrawal, S. Effect of chemical modifications of cytosine and guanine in a CpG-motif of oligonucleotides: structure-immunostimulatory activity relationships. *Bioorg. Med. Chem.* (2001), **9**: 807-813.