



Product Specifications

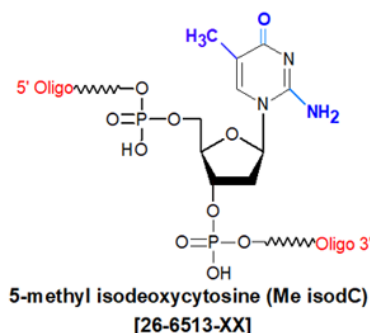
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Oligo Modifications

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

5-Me iso dC

Category	Minor Bases
Modification Code	5-Me-isodC
Reference Catalog Number	26-6513
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	303.21



5-methyl iso-deoxycytosine (5-Me-iso-dC) forms a Watson-Crick base pair with iso-dG, but has a different type of hydrogen bonding pattern than those observed for the natural base pairs A:T and C:G. Substitution of a 5-Me-iso-dC:iso-dG base pair for a C:G pair increases the T_m of the resulting duplex by ~2degC per base pair substitution (1,2). Furthermore, since 5-Me-iso-dC does not pair with dG, 5-Me-iso-dC:iso-dG can function as a stable unnatural base pair that can be used to expand the genetic code. The combination of 5-Me-iso-dC's high selectivity for iso-dG, and the resulting base pair's high thermodynamic stability, make this modified base particular attractive in the following applications:

(a) **Molecular recognition:** The 5-Me-iso-dC:iso-dG base pair has been incorporated into hybridization assays to enhance probe-target specificity and reduce spurious hybridization to non-target sequences. For example, Collins and co-workers significantly improved the sensitivity of a branched DNA quantitative hybridization assay for detecting the HIV POL sequence by incorporating ~30% 5-Me-iso-dC and iso-dG into the pre-amplifier, branched DNA (bDNA) amplifier and alkaline phosphate probe sequences used in the assay (3). Use of this strategy resulted in a significant reduction in non-specific hybridization of the above three sequence types to non-target nucleic acid sequences, and thus less amplification of background. The limits of detection of the assay were improved 10-fold, from < 500 HIV molecules/mL to < 50 molecules/mL.

(b) **qPCR and artificially expanded genetic systems:** A number of research groups have been working on optimizing PCR amplification on templates containing 5-Me-iso-dC. Such optimization is necessary to enable the full development of artificially expanded genetic systems utilizing an expanded genetic code, thereby allowing for the site-specific incorporation of novel functional components (such as unnatural amino acids) into proteins. In 2004, Johnson and co-workers observed that, by using the Klenow fragment of Taq polymerase (KF-Taq) in PCR, the fidelity of the 5-Me-iso-dC:iso-dG base pair was about 96% per amplification cycle (4). The limit in fidelity is chiefly due to the ability of iso-dG's 1,2 tautomer to mis-pair with dT. More recently, Sismour and Benner solved this problem by using 2-thio-dT (dT*) in place of dT. dT* pairs with dA, but not with iso-dG (5). Using this artificial base pair system (5-Me-iso-dC:iso-dG, dA:dT*, dC:dG) with KF-Taq, the fidelity in PCR was increased to about 98% per amplification cycle.

The achievement of high fidelity PCR with the 5-Me-iso-dC:iso-dG base pair opens the door to developing both artificially expanded genetic systems (6) and novel qPCR systems (for example, Promega's Plexor technology) based on this approach.

References

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6. Yang, Z.; Hutter, D.; Sheng, P.; Sismour, A.M.; Benner, S.A. Artificially expanded genetic information system: a new base pair with an alternative hydrogen bonding pattern, *Nucleic Acids Res.* (2006), **34**: 6095-6101.