



## Product Specifications

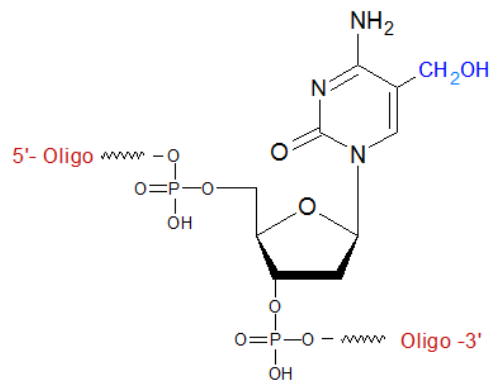
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

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

### 5-hm dC

Category	Epigenetics
Modification Code	5hm-dC
Reference Catalog Number	26-6707
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	319.21



5-Hydroxymethyl-dC  
[26-6707-XX]

5-Hydroxymethyl cytosine (5-hm-dC) is a minor DNA base; its presence in DNA strands was first observed in T-even bacteriophages (1). In such viruses, 5-hm-dC is often glycosylated, and this modified base protects phage DNA from cleavage by host restriction endonucleases after infection (2), and thus serves a **direct epigenetic role** in T-even phages. 5-hm-dC was first reported in mammalian systems in 1972, by Penn *et al.*, who found relatively high levels of this modified base in DNA extracted from the brains of adult rats, mice and frogs (~ 15% of total cytosines) (3). In a follow-up study, Penn reported the observation of a highly statistically significant increase in 5-hm-dC in rat brain tissue as rats grew from newborn (~ 8% of total cytosines) to adult (~ 18% of total cytosines), and speculated that 5-hm-dC-containing DNA, or the base itself, might be implicated in the maintenance of steady-state neuronal activity, and possibly associated with synaptosomal mitochondria (4).

However, because the presence of 5-hm-dC in mammalian brain tissue could not be confirmed in other studies conducted around the same time, the topic languished for the next 30 years. Then, in 2009, Kriaucionis and Heintz (5) reported the presence of high levels of 5-hm-dC in Purkinje neurons from mouse brain tissue, with the 5-hm-dC specifically localized to CpG regions, thus both confirming the results of Penn *et al.*'s 1972 paper and expanding on it by definitively localizing 5-hm-dC to CpG regions of DNA, suggesting that this modified base plays an important epigenetic regulatory role in the central nervous system of mammals. Shortly thereafter, Tahiliani *et al.* (6) reported that the enzyme TET1 catalyzes the conversion of 5-methyl-dC to 5-hm-dC, both *in vitro* and *in vivo*, further strengthening the case for such a role.

However, it is possible that the role of 5-hm-dC is as an intermediate in a putative (active) oxidative demethylation pathway for conversion of 5-Me-dC to dC. Demethylation of 5-Me-dC is necessary for epigenetic control of gene expression in the cell, and plays a key role in cellular reprogramming, embryogenesis, establishment of maternal and paternal methylation patterns in the genome (7), and also in certain autoimmune disorders and cancer (8). The discovery of an enzymatic pathway for conversion of 5-Me-dC to 5hm-dC, mediated by the enzyme Tet1 has spurred efforts to determine whether or not 5-hm-dC is then subsequently converted to dC through a 5-formyl-dC or 5-carboxy-dC intermediate.

In 2011, Ito and co-workers showed that Tet enzymes are able to convert 5hm-dC to both 5-formyl-dC and 5-carboxy-dC, and also observed their presence in mouse embryonic stem cells and various mouse organ tissues. Genomic content of 5hm-dC, 5-formyl-dC and 5-carboxy-dC can be modulated through overexpression or depletion of Tet proteins in these tissues (9). These experiments provide strong supporting evidence for DNA demethylation occurring via a Tet-mediated enzymatic pathway involving 5-hm-dC as a key intermediate. 5-hm-dC modified oligos can serve as important research tools for probing the DNA demethylation process.

The availability of 5-hm-dC as a phosphoramidite enables the incorporation of this modified base into synthetic oligonucleotides for use as research tools to help researchers definitively determine the role of this minor base in the biochemistry of brain and other tissues.

### 5-hydroxymethylated dC oligos

Oligos modified with 5-OH me dC (5-hmc) are totally resistant to cleavage by Hpa II restriction enzyme. Msp I and Msp JI restriction enzymes will digest these oligos to almost completion. Usually there is 25-30% resistant species remaining due to resistant protecting groups leftover during synthesis. Higher quantities of enzyme and longer incubation times (18-20 hrs) tends to increase digestion to greater than 90%. Oligos containing 5-hmc can be glucosylated by using T4  $\beta$ -glucosyltransferase and thus resistant to Msp I digestion to discern between 5-mc and 5-hmc. The 5-OH group of 5-hmc is glucosylated and becomes completely resistant to Msp I digestion.

#### References

1. Wyatt, G.R.; Cohen, S.S. A new pyrimidine base from bacteriophage nucleic acids. *Nature (London)*. (1952), **170**: 1072-1073.
2. Wiberg, J.S. Amber Mutants of Bacteriophage T4 Defective in Deoxycytidine Diphosphatase and Deoxycytidine Triphosphatase—On the Role of 5-Hydroxymethylcytosine in Bacteriophage Deoxyribonucleic Acid. *J. Biol. Chem.* (1967), **242**: 5824-5829.
3. Penn, N.W.; Suwalski, R.; O'Riley, C.; Bojanowski, K.; Yura, R. The Presence of 5-Hydroxymethylcytosine in Animal Deoxyribonucleic Acid. *Biochem. J.* (1972), **126**: 781-790.
4. Penn, N.W. Modification of Brain Deoxyribonucleic Acid Base Content with Maturation in Normal and Malnourished Rats. *Biochem. J.* (1976), **155**: 709-712.
5. Kriaucionis, S.; Heintz, N. The Nuclear Base 5-Hydroxymethylcytosine Is Present in Purkinje Neurons and the Brain. *Science (Published Online)* (16 April, 2009), DOI: **10.1126/science.1169786**: 1-3.
6. Tahiliani, M.; Koh, K.P.; Shen, Y.; Pastor, W.A.; Bandukwala, H.; Brudno, Y.; Agarwal, S.; Iyer, L.M.; Liu, D.; Aravind, L.; Rao, A. Conversion of 5-Methylcytosine to 5-Hydroxymethylcytosine in Mammalian DNA by MLL Partner TET1 *Science* (2000), **324**: 930-935.
7. Sasaki, H., Matsui, Y. Epigenetic events in mammalian germ-cell development: reprogramming and beyond. *Nat. Rev. Genet.* (2008), **9**: 129-140.
8. Richardson, B.C. Role of DNA methylation in the regulation of cell function: autoimmunity, aging and cancer. *J. Nutr.* (2002), **132(8 Suppl)**: 2401S-2405S.
9. Ito, S., Shen, L., Dai, Q., Wu, S.C., Collins, L.B., Swenberg, J.A., He, C., Zhang, Y. Tet Proteins Can Convert 5-Methylcytosine to 5-Formylcytosine and 5-Carboxylcytosine. *Science* (2011), **333**: 1300-1303.