

PRODUCT NAME		
5-hmC polyclonal antibody (rabbit)		
Full name: 5-hydroxymethylcytosine polyclonal antibody (rabbit)		
Cat. No.: C15310210-20 (CS-HMC-020) C15310210-100 (CS-HMC-100)	Type: Polyclonal	Format: 20 µl , 100 µl
Lot #: A1204-004	Source: Rabbit	Concentration: not determined

**Description:** Polyclonal antibody raised in rabbit against 5-hydroxymethylcytosine conjugated to KLH.

**Specificity:** : Human, mouse, other (wide range): positive

Applications	Suggested dilution / amount	References
ELISA	1:500	Figure 1
hMeDIP	2.5 µl/IP	Figure 2
Dot Blot	1:200	Figure 3

**Purity:** Whole antiserum from rabbit containing 0.05% Na-azide.

**Storage:** Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.

**Precautions:** This product is for research use only. Not for use in diagnostic or therapeutic procedures.

**Last data sheet update:** August 3rd, 2010

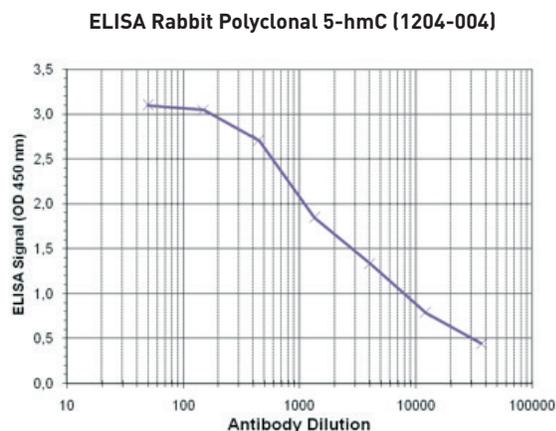
#### Target description

5-hydroxymethylcytosine (5-hmC) has been recently discovered in mammalian DNA. This results from the enzymatic conversion of 5-methylcytosine into 5-hydroxymethylcytosine by the TET family of oxygenases. Initially, the 5-hmC bases have been identified in Purkinje neurons, in granule cells and embryonic stem cells where they are present at high levels (up to 0,6% of total nucleotides in Purkinje cells). A recent report indicates that 5-hmC is abundant in brain tissue, especially in areas that are associated with higher cognitive functions.

Preliminary results indicate that 5-hmC may have important roles distinct from 5-mC. Although its precise role has still to be shown, early evidence suggests a few putative mechanisms that could have big implications in epigenetics: 5-hydroxymethylcytosine may well represent a new pathway to demethylate DNA involving a repair mechanism converting 5-hmC to cytosine and, as such open up entirely new perspectives in epigenetic studies

Due to the structural similarity between 5-mC and 5-hmC, these bases are experimentally almost indistinguishable. Recent articles demonstrated that the most common approaches (e.g. enzymatic approaches, bisulfite sequencing) do not account for 5-hmC. The development of the affinity-based technologies appears to be the most powerful way to differentially and specifically enrich 5-mC and 5-hmC sequences. The results shown here illustrate the use of this unique rabbit polyclonal antibody against 5-hydroxymethylcytosine that has been fully validated in various technologies.

Figure 1



**Determination of the 5-hmC rabbit polyclonal antibody titer**

To determine the titer, an ELISA was performed using a serial dilution of the Diagenode rabbit polyclonal antibody directed against 5-hmC in antigen coated wells. The antigen used was BSA coupled to the 5-hmC base. By plotting the absorbance against the antibody dilution, the titer of the antibody was estimated to be 1: 3,500.

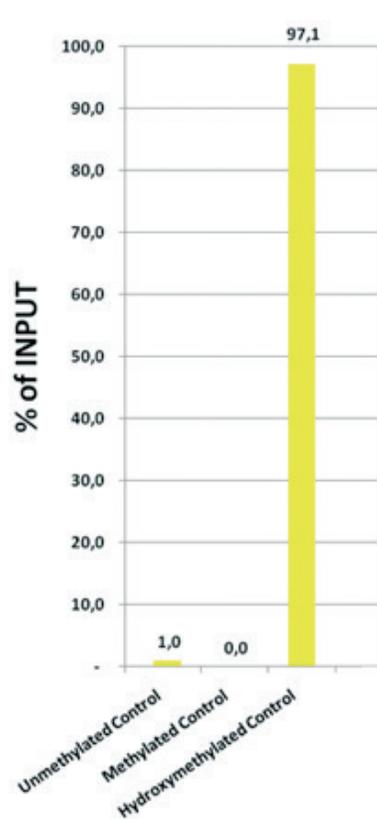
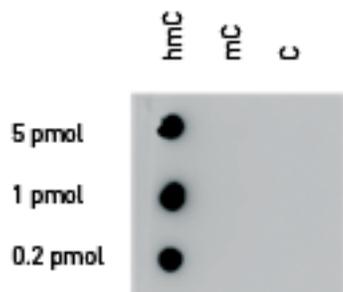


Figure 2

An hydroxymethylated DNA IP (hMeDIP) was performed using the Diagenode rabbit polyclonal antibody directed against 5-hydroxymethylcytosine (Cat. No. CS-HMC-100). The IgG isotype antibodies from rabbit (Cat. No. kch-504-250) was used as negative control. The DNA was prepared with the GenDNA module of the hMeDIP kit and sonicated with our Bioruptor® (UCD-200/300 series) to have DNA fragments of 300-500 bp. 1 µg of human Hela cells DNA were spiked with non-methylated, methylated, and hydroxymethylated fragments. The IP'd material has been analysed by qPCR using the primer pair specific for the 3 different control sequences.

The obtained results show that the Diagenode rabbit polyclonal for 5-hmC is highly specific for this base modification (no IP with non-methylated or methylated C bases containing fragments).



**Figure 3**

**Dotblot analysis of the Diagenode 5-hmC rabbit polyclonal antibody with the C, mC and hmC PCR controls**

100 to 4 ng (equivalent of 5 to 0.2 pmol of C-bases) of the hmC, mC and C PCR controls from the Diagenode "5-hmC, 5-mC & cytosine DNA Standard Pack" (Cat No. AF-101-0002) were spotted on a membrane (Amersham Hybond-N+). The membrane was incubated with the rabbit 5-hydroxymethylcytosine polyclonal antibody (dilution 1:200). The membranes were exposed for 30 seconds.