

PRODUCT NAME		
5-hmC monoclonal antibody (mouse)		
Full name: 5-hydroxymethylcytosine monoclonal antibody (mouse)		
Cat. No.: C15200200-20 (Mab-31HMC-020) C15200200-50 (Mab-31HMC-050) C15200200-100 (Mab-31HMC-100)	Type: Monoclonal Isotype: IgG1k	Format: 20 µg / 20 µl 50 µg / 50 µl 100 µg / 100 µl
Lot #: 001	Source: Mouse	Concentration: 1 µg / µl

Description: Monoclonal antibody raised in mouse against 5-hydroxymethylcytosine conjugated to BSA.

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

Applications	Suggested dilution / amount	References
hMeDIP	2.5 µg per IP	Fig 1
ELISA	1:500	Fig 2
Dot blotting	2 µg / ml	Fig 3

Purity: Affinity purified monoclonal antibody in PBS (ph 7.4) containing 0.05% sodium 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.

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. So far, 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).

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 monoclonal antibody against 5-hydroxymethylcytosine that has been fully validated in various technologies.

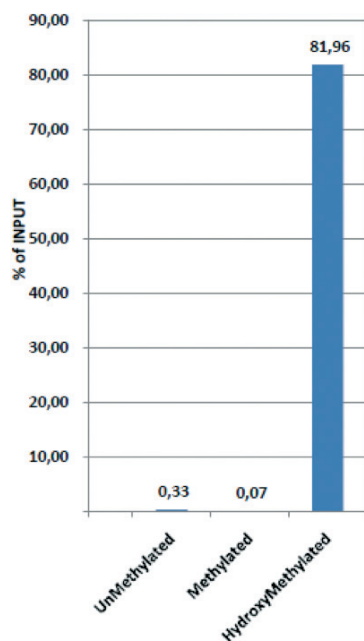


Figure 1

An hydroxymethylated DNA IP (hMeDIP) was performed using the Diagenode mouse monoclonal antibody directed against 5-hydroxymethylcytosine (Cat. No. MAb-31HMC-020, MAb-31HMC-050, MAb-31HMC-100).

The IgG isotype antibodies from mouse (Cat. No. kch-819-015) 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 PCR 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 mouse monoclonal for 5-hmC is highly specific for this base modification (no IP with non-methylated or methylated C bases containing fragments).

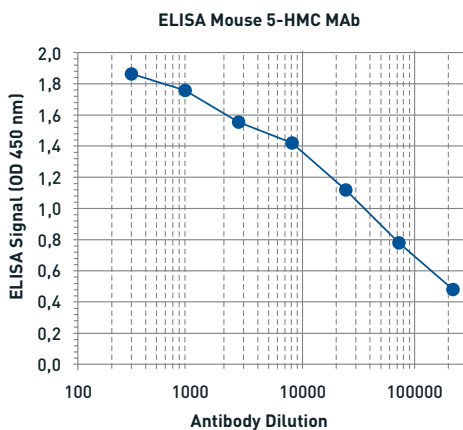
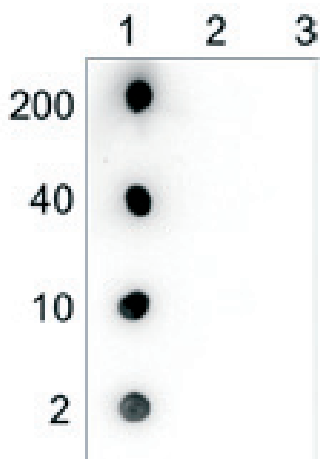


Figure 2
Determination of the 5-hmC mouse monoclonal antibody titer

To determine the titer, an ELISA was performed using a serial dilution of the Diagenode mouse monoclonal antibody directed against 5-hmC (Cat No. MAb-31HMC-050, MAb-31HMC-100) in antigen coated wells. The antigen used was KHL coupled to 5-hmC base. By plotting the absorbance against the antibody dilution, the titer of the antibody was estimated to be 1:40,000.

Figure 3



Dotblot analysis of the Diagenode 5-hmC mouse monoclonal antibody with the C, mC and hmC PCR controls
200 to 2 ng (equivalent of 10 to 0.1 pmol of C-bases) of the hmC (1), mC (2) and C (3) PCR controls from the Diagenode "5-hmC, 5-mC & cytosine DNA Standard Pack" (Cat No. AF-101-0020) were spotted on a membrane (Amersham Hybond-N+). The membrane was incubated with 2 µg/ml of the mouse 5-hydroxymethylcytosine monoclonal antibody (dilution 1:500). The membranes were exposed for 30 seconds.