diagenode
A Hologic Company

TECHNICAL DATASHEET

PRODUCT NAME **5-hmC polyclonal antibody**		
(Full name : 5-hydroxymethylcytosine polyclonal antibody)		
Cat. No. **C15410205-20** (pAb-HMC-020) **C15410205-50** (pAb-HMC-050)	**Type:** Polyclonal	**Size:** 20 µg/6 µl 50 µg/14.3 µl
Lot #: A1203-0041	**Source:** Rabbit	**Concentration:** 3.5 µg/µl

Product description: Polyclonal antibody raised in rabbit against 5-hydroxymethylcytosine conjugated to KLH.

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

Applications	Suggested dilution	References
hMeDIP*	3.5 µg/IP	Fig 1
ELISA	1:100 - 1:500	Fig 2
Dot blot	1:1,000	Fig 3

*Please note that of the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 µg per IP

Purity: Protein G purified polyclonal antibody in PBS containing 0.05% azide and 0.05% ProClin 300.

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: May 11, 2011

Target description

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

Early evidence suggests that 5-hmC may represent an intermediate in a new pathway which demethylates DNA, converting 5-mC to cytosine. Although its precise role has still to be shown, recent results indicate, however, that 5-hmC may play important roles distinct from 5-mC. This may open up entirely new perspectives in epigenetic studies.

Due to their structural similarity, 5-mC and 5-hmC 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 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 validation of this unique rabbit polyclonal antibody against 5-hydroxymethylcytosine in various applications.

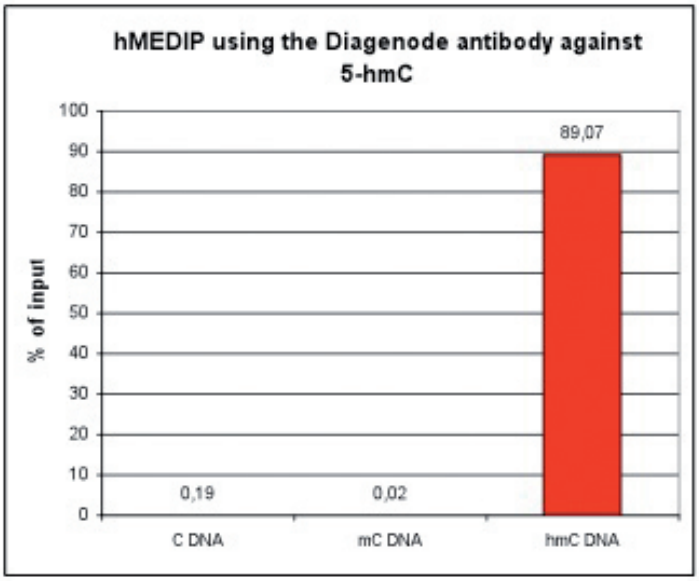

Figure 1

hMeDIP results obtained with the Diagenode antibody directed against 5-hmC

hMeDIP (hydroxymethylated DNA IP) was performed using the Diagenode antibody against 5-hydroxymethylcytosine (Cat. No. pAb-HMC-050). DNA from mouse ES cells was prepared with the GenDNA module of the hMeDIP kit and sonicated with our Bioruptor® (UCD-200/300 series) to obtain DNA fragments of 300-500 bp. One µg of sheared DNA was spiked with the unmethylated (C) methylated (mC), and hydroxymethylated (hmC) controls from the Diagenode "5-hmC, 5-mC & cytosine DNA Standard Pack for hMeDIP" (Cat No. AF-107-0040). hMeDIP was performed with 3.5 µg of the rabbit 5-hmC antibody and the IP'd DNA was analysed by qPCR using primers specific for the 3 different control sequences.

Figure 1 shows that the Diagenode rabbit polyclonal antibody against 5-hmC is highly specific for the 5-hmC base modification (no IP with non-methylated or methylated C control fragments).

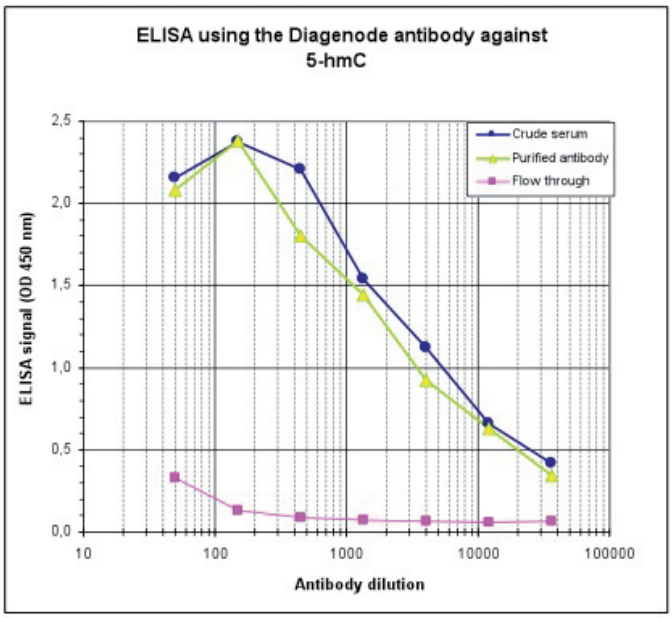

Figure 2
Determination of the antibody titer
To determine the titer, an ELISA was performed using a serial dilution of the Diagenode antibody directed against 5-hmC (cat. No. pAb-HMC-050), crude serum and flow through, in antigen coated wells. The antigen used was the 5-hmC base coupled to BSA. By plotting the absorbance against the antibody dilution, the titer of the antibody was estimated to be 1:2,800.

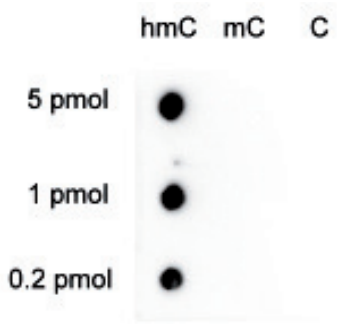

Figure 3
Dot blot analysis using the Diagenode antibody directed against 5-hmC
To demonstrate the specificity of the Diagenode antibody against 5-hmC (cat. No. pAb-HMC-050), a Dot blot analysis was performed using the hmC, mC and C controls from the Diagenode "5-hmC, 5-mC & cytosine DNA Standard Pack" (Cat No. AF-101-0002). One hundred to 4 ng (equivalent of 5 to 0.2 pmol of C-bases) of the controls were spotted on a membrane (Amersham Hybond-N+). The antibody was used at a dilution of 1:1,000. Figure 3 shows a high specificity of the antibody for the hydroxymethylated control.