diagenode
A Hologic Company

TECHNICAL DATASHEET

5-hmC monoclonal antibody (rat)

Cat. No. C15220001
Type: Monoclonal
Isotype: IgG2a
Source: Rat
Lot #: 002
Size: 20 µg - 50 µg - 100 µg
Concentration: 1 µg/µl

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

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

Applications

	Suggested dilution	Results
hMeDIP	2.5 µg per IP	Fig 1
ELISA	1:1000	Fig 2
Dot blotting	1:500 (4 µg/ml)	Fig 3, 4

Target description

5-hydroxymethylcytosine (5-hmC) has been recently discovered in mammalian DNA by two US groups (Kriaucionis & Heintz, Science, 2009 and Tahiliani et al., Science, 2009). 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.

Results

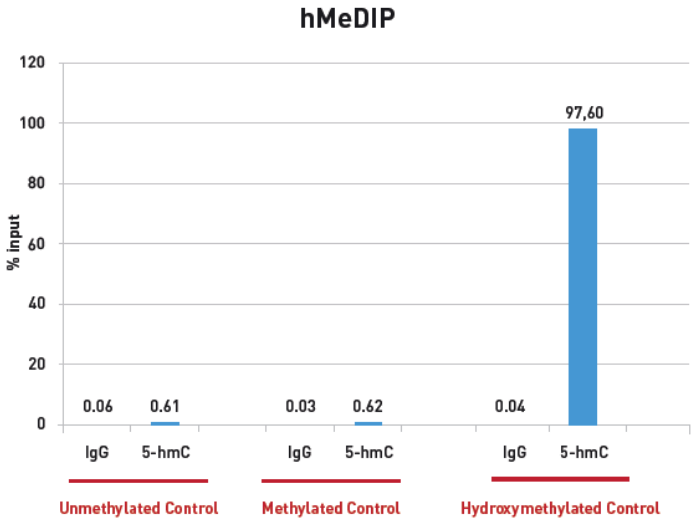

Figure 1. Hydroxymethylated DNA IP results obtained with our hMeDIP kit (Cat. No.C02010030).

Hydroxymethylated DNA IP (hMeDIP) assays were performed using the Diagenode hMeDIP kit. This kit includes: the monoclonal antibody against 5-hydroxymethylcytosine (Cat. No. C15220001), 5-hmC, 5-mC & cytosine DNA standards & Rat IgG (Cat. No. C15420001). The DNA was prepared with the GenDNA module and sonicated with our Bioruptor® (UCD-200/300 series) to obtain DNA fragments of 300-500 bp. 1 µg of mouse ES cells DNA was spiked with 0.025 ng of each DNA standard. The IP'd material has been analysed by qPCR using the primer pairs specific to the control sequences.

The obtained results are as follows:

- hMeDIP on unmethylated control
  - with Rat IgG as negative control (0.06%, almost no recovery)
  - with 5-hmC antibody (0.61%, almost no recovery)
- hMeDIP on methylated control
  - with Rat IgG as negative control (0.03%, almost no recovery)
  - with 5-hmC antibody (0.62%, almost no recovery)
- hMeDIP on hydroxymethylated control
  - with Rat IgG as negative control (0.04%, almost no recovery)
  - with 5-hmC (97.60% recovery, almost full recovery)

These results clearly demonstrate the high specificity and efficiency of the 5-hydroxymethylcytosine monoclonal antibody.

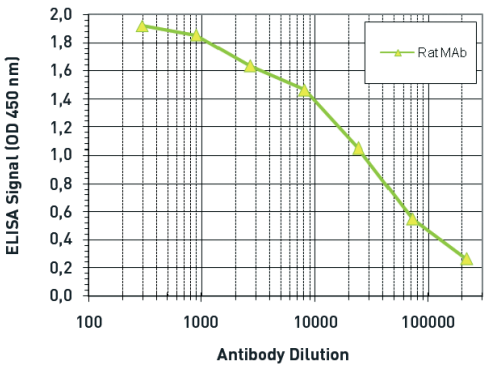

Figure 2. Determination of the 5-hmC rat monoclonal antibody titer

To determine the titer, an ELISA was performed using a serial dilution of the Diagenode monoclonal antibody directed against 5-hmC (Cat No. C15220001) in antigen coated wells. The antigen used was a 5-hmC base coupled to KHL. By plotting the absorbance against the antibody dilution, the titer of the antibody was estimated to be 1:25,000.

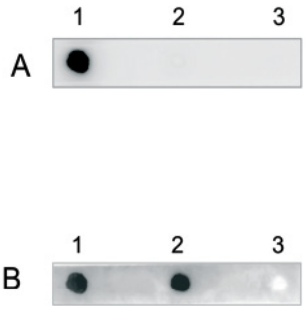

Figure 3. Dotblot analysis of the Diagenode 5-hmC and 5-mC monoclonal antibodies with the C, mC and hmC PCR controls

Figure 3A: Approximately 200 ng, equivalent 10 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. C02040010) were spotted on a membrane (Amersham Hybond-N+). The membrane was incubated with 5-hydroxymethylcytosine rat monoclonal antibody (dilution 1:500 ; 4 µg/ml final concentration), followed by an HRP conjugated anti-rat secondary antibody. The membrane was exposed during 30 seconds.

Figure 3B: Incubation of the same membrane with the 5-methylcytosine mouse monoclonal antibody (Cat. No. C15200200) (dilution 1:250). Note that the membrane was not stripped after the 5-hmC incubation. The left spot represents the remaining hmC signal. This result confirms that an equal amount of mC bases was spotted at position 2.

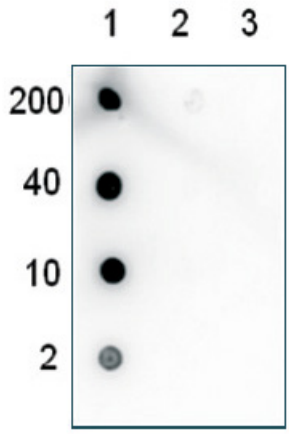

Figure 4. Dotblot analysis of the Diagenode 5-hmC rat monoclonal antibody with the C, mC and hmC PCR controls

200 to 2 ng (equivalent of 10 to 0.1 pmol of C-base) of the hmC (1), mC (2) and C (3) PCR controls from the Diagenode « 5-hmC, 5-mC & cytosine DNA Standard Pack" (Cat. No. C02040010) were spotted on a membrane (Amersham Hybond-N+). The membrane was incubated with 4 µg/ml (dilution 1:500) of the 5-hydroxymethylcytosine rat monoclonal antibody, followed by an HRP conjugated anti-rat secondary antibody. The membrane was exposed for 30 seconds.

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