

5-hmC Antibody

ALTERNATE NAMES: 5-hydroxymethylcytosine

CATALOG #: 6830-50

AMOUNT: 50 µl

HOST/ISOTYPE: Rabbit

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

FORM: Liquid

FORMULATION: In PBS containing 0.05% azide.

PURIFICATION: Whole antiserum from rabbit

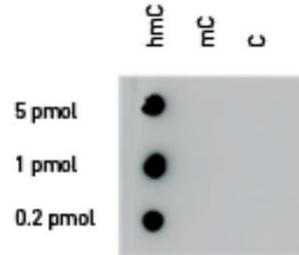
SPECIES REACTIVITY: Human, mouse and wide range of samples.

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

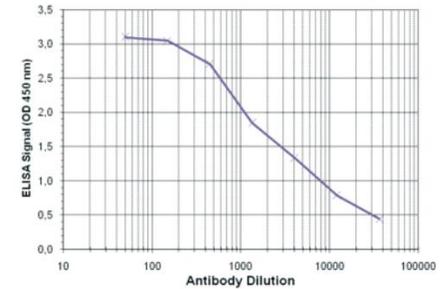
DESCRIPTION: 5-hydroxymethylcytosine (5-hmC) has been recently discovered in mammalian DNA. This results from the enzymatic conversion of 5-methylcytosine into 5-hydroxymethylcytosine by the TET family of oxygenases. Initially, the 5-hmC bases have been identified in Purkinje neurons, in granule cells 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.

APPLICATION: hMeDIP: 2.5 µg/IP, Dot Blot: 1:200, ELISA: 1:500.

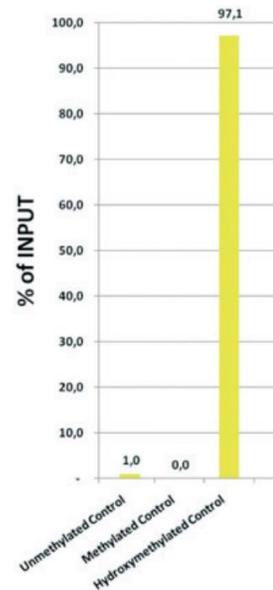
Note: This information is only intended as a guide. The optimal dilutions must be determined by the user.



100 to 4 ng (equivalent of 5 to 0.2 pmol of C-bases) of hmC, mC and C PCR controls were spotted on a membrane. The membrane was incubated with the rabbit 5-hydroxymethylcytosine polyclonal antibody (dilution 1:200). The membranes were exposed for 30 seconds.



To determine the titer, an ELISA was performed using a serial dilution of the antibody. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution the titer of the antibody was estimated to be 1:3500.



A hydroxymethylated DNA IP (hMeDIP) was performed using the antibody. The IgG isotype antibodies from rabbit was used as negative control. 1 µg of human HeLa cell's 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 polyclonal antibody for 5-hmC is highly specific for this base modification (no IP with non-methylated or methylated C bases containing fragments).

RELATED PRODUCTS:

- 5-Methylcytosine hydrochloride (Cat # 2305-50, -250)

FOR RESEARCH USE ONLY! Not to be used on humans.