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## Data Sheet

### Anti-5-hmC polyclonal antibody (rabbit)

Catalog #: 25205

<b>Lot #:</b> 150130	<b>Host Species:</b> Rabbit
<b>Conc.:</b>	<b>Species Reactivity:</b> Human, Mouse, broad range
<b>Size:</b> 100 µl	<b>Immunogen:</b> Nucleotide
<b>Clonality:</b> Polyclonal	<b>Purification:</b> Whole serum

**Description:** Polyclonal antibody raised in rabbit against 5-hydroxymethylcytosine (5-hmC) conjugated to KLH

**Background:** 5-hydroxymethylcytosine (5-hmC) results from the enzymatic conversion of 5-methylcytosine into 5-hydroxymethylcytosine by the TET family of iron-dependent oxygenases. 5-hmC bases were recently discovered in mammalian DNA, 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). Recent reports indicate 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 5-hydroxymethylcytosine may represent a new and unique pathway to demethylate DNA involving a repair mechanism converting 5-hmC to cytosine. Due to the structural similarity between 5-mC and 5-hmC, these bases are experimentally almost indistinguishable. The most commonly used methodologies (e.g. enzymatic approaches, bisulfite sequencing) do not distinguish 5mC from 5-hmC. The development of specific antibodies appears to be the most powerful way to distinguish and specifically enrich for 5-mC and 5-hmC sequences.

**Formulation:** Whole antiserum from rabbit containing 0.05% azide

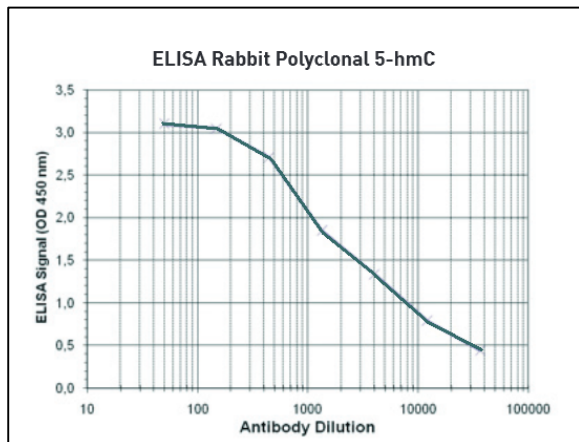
**Applications:** hMeDIP (2.5 µg/IP), ELISA (1:500), DB (1:200)

**Storage/Stability:** Store at -80 °C for up to 2 years. Centrifuge after first thaw to maximize product recovery. Aliquot to avoid repeated freeze/thaw cycles. Aliquots may be stored at -20 °C for at least one month.

**Warnings:** Avoid freeze/thaw cycles

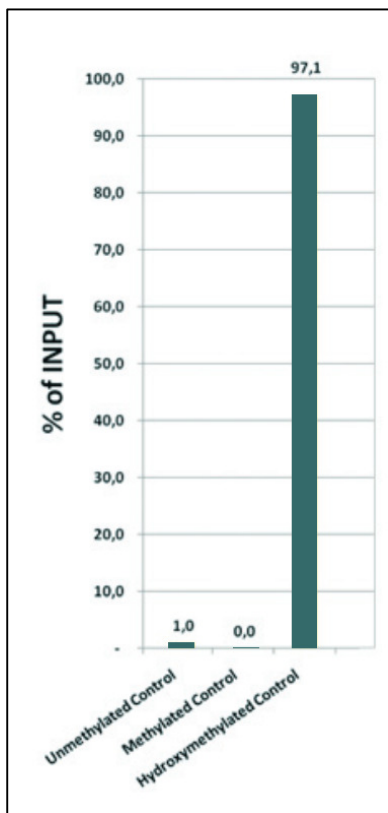
**Notes:** The optimal antibody concentration should be determined by the end-user.

**Quality Assurance:**



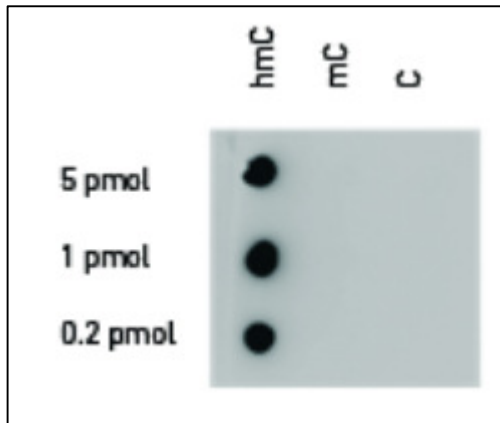
**Determination of the 5-hmC rabbit polyclonal antibody titer.**

To determine the titer, an ELISA was performed using a serial dilution of the 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.



**An hydroxymethylated DNA IP (hMeDIP) was performed using the rabbit polyclonal antibody directed against 5-hydroxymethylcytosine (Cat. No. 25205).**

The IgG isotype antibodies from rabbit were used as negative control. The DNA was prepared with the GenDNA module of the hMeDIP kit and sonicated wto have DNA fragments of 300-500 bp. 1 µg of human HeLa cells DNA were spiked with non-methylated, methylated, and hydroxymethylated fragments. The immunoprecipitated material has been analysed by qPCR using the primer pair specific for the 3 different control sequences. The obtained results show that the rabbit polyclonal for 5-hmC is highly specific for this base modification (no IP with non-methylated or methylated C bases containing fragments).



**Dot blot analysis of the 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 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.