

## 5-Hydroxymethylcytosine (5-hmC) antibody (pAb)

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**Catalog Nos:** 39791, 39092, 39792

**RRID:** AB\_2630381

**Isotype:** IgG

**Application(s):** DB, IF, IHC, MeDIP

**Reactivity:** Human, Mouse, Not Species Specific

**Quantities:** 100 µg, 50 µg, 10 µg

**Purification:** Protein A Chromatography

**Host:** Rabbit

**Concentration:** 1 µg/µl

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**Background:** Active Motif offers two polyclonal antibodies that recognize 5-hydroxymethylcytosine, a whole serum version (39769) and a purified IgG version (39791). Both are validated for use in methyl DNA immunoprecipitation (MeDIP). For customers that require the ability to quantitate the amount of IgG in the MeDIP reaction, the purified IgG version (39791) is recommended. The whole serum version (39769) is very high titre and should be used carefully (0.1 - 0.5 µl per IP) as not to generate high non-specific background. The whole serum version (39769) has been used successfully in immunofluorescence (IF, Ito *et al*, 2010), and the purified IgG version (39791) is likely to work in this application as well.

DNA methylation is an epigenetic event in which DNA methyltransferases (DNMTs) catalyze the reaction of a methyl group to the fifth carbon of cytosine in a CpG dinucleotide. This modification helps to control gene expression and is also involved in genomic imprinting, while aberrant DNA methylation is often associated with disease. 5-methylcytosine is a modified base that is found in the DNA of plants and vertebrates. A second type of DNA methylation exists, 5-hydroxymethylcytosine (5-hydroxy methylcytosine, 5-hmC). This results from the enzymatic conversion of 5-methylcytosine into 5-hydroxymethylcytosine by the TET family of cytosine oxygenases. This antibody was developed specifically to distinguish 5-hydroxymethylcytosine from 5-methylcytosine as conventional methods (enrichment by antibody or methyl DNA binding protein, enzymatic digestion and bisulfite sequencing) cannot do so. It is possible that 5-hydroxymethylcytosine (5-hmC) represents a pathway to demethylate DNA, as 5-hydroxymethylcytosine is repaired as mismatched DNA and replaced with unmethylated cytosine.

**Immunogen:** This 5-Hydroxymethylcytidine antibody was raised against 5-hydroxymethylcytidine conjugated to KLH and recognizes 5-hydroxymethylcytosine.

**Buffer:** PBS pH 7.5 containing 30% glycerol and 0.035% sodium azide. Sodium azide is highly toxic.

### Application Notes:

Applications Validated by Active Motif:

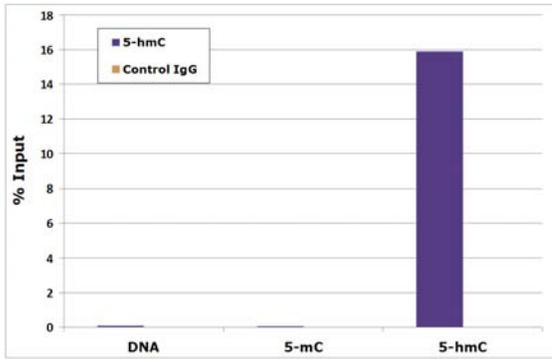
MeDIP: 1 - 2 µg per IP

IHC (FFPE): 1 µg/ml dilution

DB: 0.2 µg/ml dilution

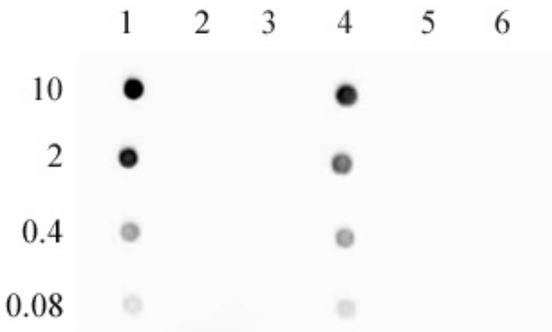
**Storage and Guarantee:** Some products may be shipped at room temperature. This will not affect their stability or performance. Avoid repeated freeze/thaw cycles by aliquoting items into single-use fractions for storage at -20°C for up to 2 years. Keep all reagents on ice when not in storage. This product is guaranteed for 12 months from date of receipt.

This product is for research use only and is not for use in diagnostic procedures.



#### 5-Hydroxymethylcytidine antibody tested by Methyl DNA immunoprecipitation.

DNA (25 pg) derived from the promoter of the APC gene was spiked into 500 ng of human genomic DNA and subjected to the MeDIP procedure using 2 µg of 5-Hydroxymethylcytidine antibody (5hmC, maroon bars) or 2 µg of control rabbit IgG (IgG, blue bars). Real time quantitative PCR was performed on the immunoprecipitated DNA and results plotted as % of input DNA. The spiked APC DNA contained either no methylation (DNA), 5-methylcytosine methylation (5-mC) or 5-hydroxymethylcytosine methylation (5-hmC). The 5-hmC antibody shows a 650-fold enrichment for 5-hmC DNA compared to 5-mC DNA or unmethylated DNA.



#### 5-Hydroxymethylcytidine antibody tested by dot blot analysis.

DNA from the Methylated DNA Standard Kit (Catalog No. 55008) were spotted (indicated in ng on the left) on to a positively charged nylon membrane and blotted with 5-Hydroxymethylcytidine antibody recognizing 5-hydroxymethylcytosine (1 µg/ml dilution).

Lane 1: double-stranded DNA 5-hydroxymethylcytosine.

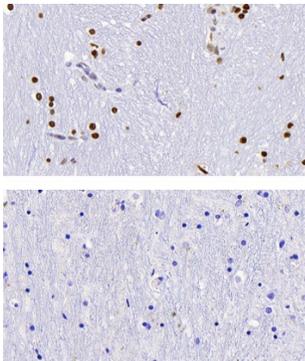
Lane 2: double-stranded DNA containing 5-methylcytosine.

Lane 3: double stranded unmethylated DNA.

Lane 4: single-stranded DNA containing 5-hydroxymethylcytosine.

Lane 5: single-stranded DNA containing 5-methylcytosine.

Lane 6: single stranded unmethylated DNA.



#### 5-Hydroxymethylcytosine (5-hmc) antibody (pAb) tested by Immunohistochemistry

Punctate nuclear staining pattern is detected in Formalin-fixed, paraffin-embedded tissue sections from human substantia nigra (midbrain). Top Panel: 5-mC antibody at 1:1000 dilution. Bottom Panel: No primary antibody (2nd step antibody alone)