

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

**Catalog Nos:** 39999, 40900, 40000

**RRID:** AB\_2566808

**Clone:** 59.1

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

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

**Quantities:** 100 µg, 50 µg, 10 µg

**Purification:** Protein G Chromatography

**Host:** Mouse

**Isotype:** IgG2a

**Background:** In addition to this monoclonal, Active Motif offers two polyclonal antibodies that recognize 5-hydroxymethylcytosine, a whole serum version (39769) and a purified IgG version (39791). All 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, we recommend either this monoclonal or the purified IgG polyclonal (39791). 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-Hydroxymethylcytosine antibody was raised against 5-hydroxymethylcytidine conjugated to KLH and recognizes 5-hydroxymethylcytosine.

**Buffer:** Purified IgG in PBS, 30% glycerol and 0.035% sodium azide. Sodium azide is highly toxic.

### Application Notes:

Applications Validated by Active Motif:

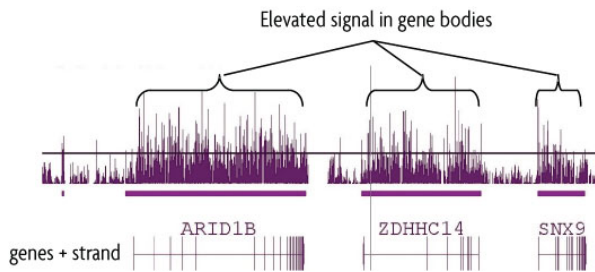
MeDIP: 1 - 2 µg per IP

MeDIP-chip: 10 µg per IP

DB: 0.2 µg/ml dilution

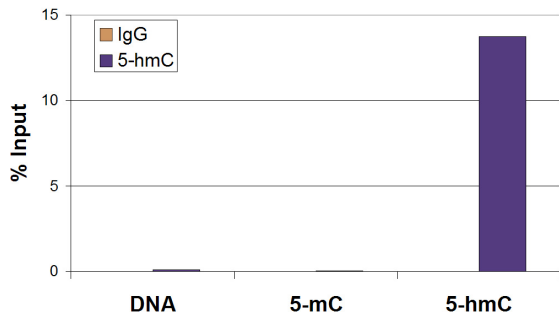
**Storage and Guarantee:** Some products may be shipped at room temperature. This will not affect their stability or performance. Avoid repeated freeze/thaw cycles by aliquoting items into single-use fractions for storage at -20°C for up to 2 years. Keep all reagents on ice when not in storage. This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



#### hMeDIP-chip performed on human brain DNA using 5-Hydroxymethylcytosine (5-hmC) antibody.

Human brain DNA (2 µg) was immunoprecipitated with 10 µg of 5-Hydroxymethylcytosine antibody. Following hMeDIP, the DNA was amplified, labeled and hybridized to an Affymetrix Human Tiling 2.0R Array. Shown is a region from chromosome 6q containing the ARID1B, ZDHHC14 and SNX9 genes. The results show that 5-hydroxymethylcytosine is enriched primarily in the coding regions of genes, rather than in the gene promoter or regulatory regions.

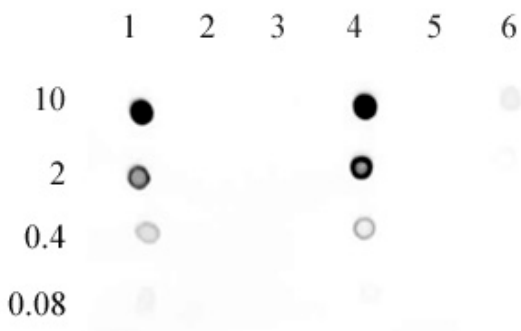


#### 5-Hydroxymethylcytosine (5-hmC) antibody tested by Methyl DNA immunoprecipitation.

DNA (25 µg) 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-Hydroxymethylcytosine 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).

#### 5-Hydroxymethylcytosine (5-hmC) antibody (mAb) tested by dot blot analysis.

DNA samples were spotted (indicated in ng on the left) on to a positively charged nylon membrane and blotted with 5-Hydroxymethylcytosine antibody at a 0.2 mg/ml dilution.



Lane 1: double-stranded DNA containing 5-hydroxymethylcytosine.

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

Lane 3: unmethylated double-stranded DNA.

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

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

Lane 6: unmethylated single-stranded DNA.

#### Dot blot of 5-Hydroxymethylcytosine (5-hmC) mAb.

Dot blot analysis was used to confirm the specificity of 5-Hydroxymethylcytosine antibody for 5-hydroxymethylcytosine. 10 ng, 2 ng, and 0.4 ng of single-stranded 38 nt DNA oligonucleotides were spotted onto nitrocellulose and probed with the antibody at 0.2mg/mL. Lane 1: oligo containing unmodified cytosine. Lane 2: oligo containing 5-methylcytosine. Lane 3: oligo containing 5-hydroxymethylcytosine. Lane 4: oligo containing 5-formylcytosine. Lane 5: oligo containing 5-carboxylcytosine.

