

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

Catalog No: 39791, 39792

Isotype: IgG

Application(s): DB, MeDIP

Reactivity: Human, Mouse, Other (Wide Range)

Purification: Protein A Chromatography

Host: Rabbit

Concentration: 1 µg/µl

Quantity: 100 µg, 10 µg

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:

Validated Applications:

MeDIP: 1 - 2 µg per IP

DB: 0.2 µg/ml dilution

Published Applications:

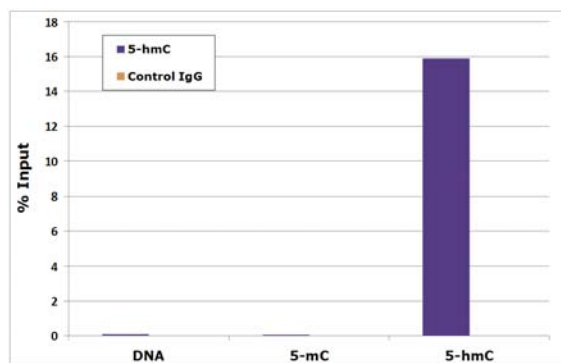
MeDIP-Seq

MeDIP

See references for more information. Individual optimization may be required.

Storage and Guarantee: Antibodies in solution can be stored at -20°C for 2 years. Avoid repeated freeze/thaw cycles and keep on ice when not in storage. This product is guaranteed for 6 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.

