

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

Catalog No: 39769, 39770

Isotype: Serum

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

Reactivity: Human, Mouse, Other (Wide Range)

Purification: None

Host: Rabbit

Volume: 100 µl, 10 µl

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 who must 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, so should be used carefully (0.1 - 0.5 µl per IP) to prevent non-specific background. The whole serum version (39769) has been used successfully in immunofluorescence (IF, Ito *et al*, 2010); the purified IgG version (39791) is likely to work in IF 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: Rabbit serum containing 30% glycerol and 0.035% sodium azide. Sodium azide is highly toxic.

Application Notes:

Validated Applications:

MeDIP: 0.1 - 0.5 µl per IP

DB: 1:10,000 dilution

Published Applications:

MeDIP

MeDIP-Seq

ICC/IF

IHC

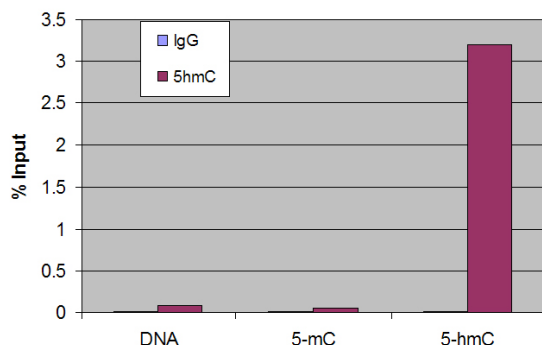
FC

DB

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

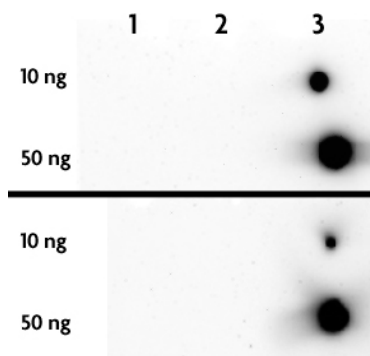
Storage and Guarantee: Antibodies in solution can be stored at -20°C for 2 years. Avoid repeated freeze/thaw cycles and keep on ice when not in storage. This product is guaranteed for 6 months from date of receipt.

Application Key: ChIP = Chromatin Immunoprecipitation; FACS = Flow Cytometry; IF = Immunofluorescence; IHC = Immunohistochemistry; IP = Immunoprecipitation; WB = Western Blot



5-Hydroxymethylcytosine (5-hmC, 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 1 µl of 5-Hydroxymethylcytidine antibody (5hmC, maroon bars) or 1 µl 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, 5-hydroxymethylcytidine) antibody tested by dot blot analysis.

DNA samples (10 ng or 50 ng as indicated) were spotted onto positively charged nylon membrane and blotted with 5-Hydroxymethylcytidine antibody at a dilution of 1:10,000.

Top Panel: Double stranded DNA.

Bottom Panel: Single stranded DNA.

Lane 1: Unmethylated DNA.

Lane 2: DNA containing 5-methylcytosine.

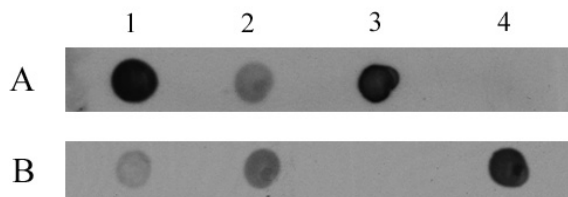
Lane 3: DNA containing 5-hydroxymethylcytosine.

5-Hydroxymethylcytosine (5-hmC, 5-hydroxymethylcytidine) antibody tested by dot blot analysis.

DNA samples were spotted onto positively charged nylon membrane and blotted with antibodies as indicated.

Panel A: 5-Hydroxymethylcytidine antibody recognizing 5-hydroxymethylcytosine (1:10,000 dilution).

Panel B: 5-Methylcytidine antibody (1:1,000 dilution).



Lane 1: DNA derived from mouse embryonic stem cells (150 ng).

Lane 2: DNA derived from mouse spleen (600 ng).

Lane 3: 27 base oligonucleotide containing 5-hydroxymethylcytosine (1.2 ng).

Lane 4: 33 base oligonucleotide containing 5-methylcytosine (2000 ng).