5-Hydroxymethylcytosine (5-hmC) antibody (rAb)

Catalog No: 91309

Clone: RM236
Isotype: IgG
Application(s): DB, IF, IHC
Reactivity: Human, Not Species Specific

Quantity: 50 µg
Purification: Protein A Chromatography
Host: Rabbit
Concentration: 1 µg/µl

Background: 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: BSA-conjugated 5-hydroxymethylcytosine.

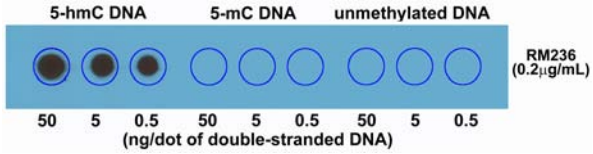
Buffer: PBS with 50% glycerol, 1% BSA, and 0.09% sodium azide.

Application Notes:
Validated Applications:
DB: 0.2 - 1 µg/ml
IHC: 0.1 - 1 µg/ml
ICC: 0.5 - 2 µg/ml
MeDIP: 0.2 - 2 µg/ml

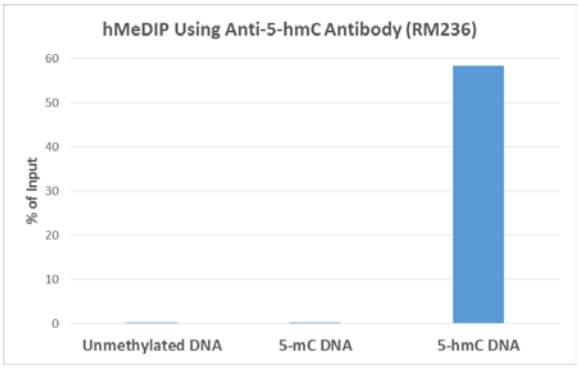
References: 5-Hydroxymethylcytosine (5-hmC) antibody (rAb) has been cited in one or more publications. To see an up-to-date listing of papers that describe its use, please go to www.activemotif.com/catalog/details/91309.

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.

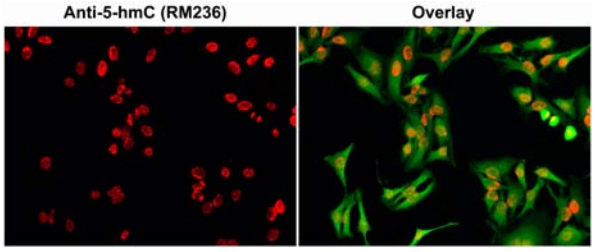
This product is for research use only and is not for use in diagnostic procedures.



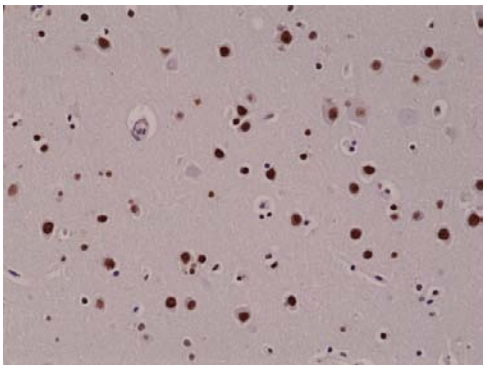
Dot blot of 5-Hydroxymethylcytosine (5-hmC) antibody (rAb). Membrane was pre-spotted with 50, 5, and 0.5 ng/dot of double stranded 5-Hydroxymethylcytosine (5-hmC) DNA, 5-Methylcytosine (5-mC) DNA, and unmethylated DNA. The pre-spotted membrane was then blotted with antibody at 0.2 µg/ml.



MeDIP of 5-Hydroxymethylcytosine (5-hmC) antibody (rAb). MeDIP was performed using 5-Hydroxymethylcytosine antibody (Clone RM236) at a 10:1 DNA:Ab ratio. 1 ng of unmethylated, 5-Methylcytosine (5-mC) or 5-Hydroxymethylcytosine (5-hmC) DNA standard (897 bp) was spiked in 1 µg of genomic DNA isolated from HeLa cells as the control. Realtime PCR was then performed to determine the capture of DNA standard as in % of input.



Immunofluoresence staining of HeLa cells using 5-Hydroxymethylcytosine antibody (Clone RM236) at 0.5ug/mL (red). Actin filaments are labeled with fluorescein phalloidin (green). HeLa cells were fixed with 4% paraformaldehyde and permeabilized with methanol (−20°C) before treatment with 2 N HCl for 30 min at 37 °C to denature DNA.



Immunohistochemical staining of formalin fixed, paraffin embedded human brain tissue sections, using
5-Hydroxymethylcytosine antibody (Clone RM236) at 1 µg/ml.