Revision 4

Store at -20C

#51660

5-Hydroxymethylcytosine (5-hmC) (HMC31) Mouse mAb

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	Source/Isotype:
IF-IC, Dot Blot	All	Endogenous	Mouse IgG1

Product Usage Information

Application	Dilution
Immunofluorescence (Immunocytochemistry)	1:400
DNA Dot Blot	1:1000

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity/Sensitivity

5-Hydroxymethylcytosine (5-hmC) (HMC31) Mouse mAb recognizes endogenous levels of 5-hmC; however many cells and tissues contain very low levels of 5-hmC that may fall below the detection limits of this antibody. This antibody has been validated using ELISA, dot blot, and MeDIP assays and shows high specificity for 5-hmC.

Source / Purification

Monoclonal antibody is produced by immunizing animals with 5-hydroxymethylcytidine.

Background

Methylation of DNA at cytosine residues is a heritable, epigenetic modification that is critical for proper regulation of gene expression, genomic imprinting, and mammalian development (1,2). 5-methylcytosine is a repressive epigenetic mark established *de novo* by two enzymes, DNMT3a and DNMT3b, and is maintained by DNMT1 (3, 4). 5-methylcytosine was originally thought to be passively depleted during DNA replication. However, subsequent studies have shown that Ten-Eleven Translocation (TET) proteins TET1, TET2, and TET3 can catalyze the oxidation of methylated cytosine to 5-hydroxymethylcytosine (5-hmC) (5). Additionally, TET proteins can further oxidize 5-hmC to form 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC), both of which are excised by thymine-DNA glycosylase (TDG), effectively linking cytosine oxidation to the base excision repair pathway and supporting active cytosine demethylation (6,7).
TET protein-mediated cytosine hydroxymethylation was initially demonstrated in mouse brain and embryonic stem cells (5, 8). Since then this modification has been discovered in many tissues, with the highest levels found in the brain (9). While 5-fC and 5-caC appear to be short-lived intermediate species, there is mounting evidence showing that 5-hmC is a distinct epigenetic mark with various unique functions (10,11). The modified base itself is stable in vivo and interacts with various readers including MeCP2 (11,12). The global level of 5-hmC increases during brain development and 5-hmC is enriched at promoter regions and poised enhancers. Furthermore, there is an inverse correlation between levels of 5-hmC and histone H3K9 and H3K27 trimethylation, suggesting a role for 5-hmC in gene activation (12). Lower amounts of 5-hmC have been reported in various cancers including myeloid leukemia and melanoma (13,14).

Background References

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Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Applications Key

IF-IC: Immunofluorescence (Immunocytochemistry) **Dot Blot:** DNA Dot Blot

Cross-Reactivity Key

All: All Species Expected

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