PRODUCT INFORMATION

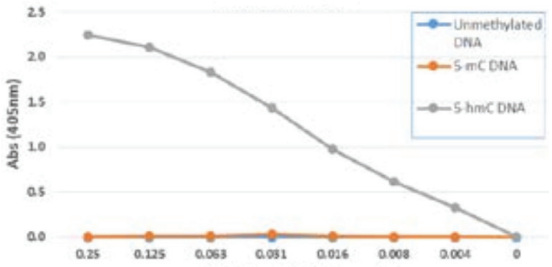
Anti-5-hydroxy Methylcytosine Rabbit Monoclonal Antibody (Clone RM236)

Item No. 20723

Overview and Properties

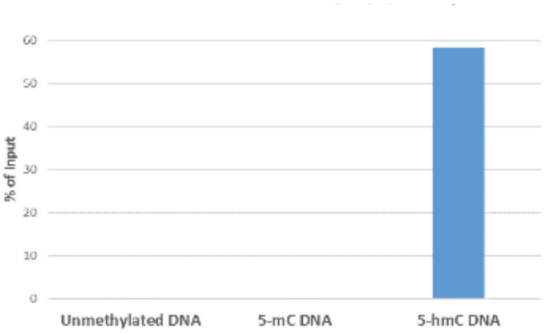
Contents:	This vial contains 50 µg of protein A affinity-purified antibody from an animal origin free culture supernatant.
Synonym:	5-hmC
Immunogen:	BSA-conjugated 5-hydroxy methylcytosine
Cross Reactivity:	(+) 5-hydroxy methylcytosine in both single-stranded and double-stranded DNA; (-) Non-methylated cytosine, methylcytosine in DNA
Species Reactivity:	(+) All species
Form:	Liquid
Storage:	-20°C (as supplied)
Stability:	As supplied, 1 year from the QC date provided on the Certificate of Analysis, when stored properly
Storage Buffer:	50% glycerol/PBS with 1% BSA and 0.09% sodium azide
Clone:	RM236
Host:	Rabbit
Isotype:	IgG
Applications:	Dot blot (DB), ELISA, Hydroxymethylated DNA immunoprecipitation (hMeDIP), Immunocytochemistry (ICC), and Immunohistochemistry (IHC); the recommended starting dilution for DB is 0.2 - 1 µg/ml, 0.1 - 1 µg/ml for ELISA and IHC, 0.2 - 2 µg/ml for hMeDIP, and 0.5 - 2 µg/ml for ICC. Other applications were not tested, therefore optimal working concentration/dilution should be determined empirically.

Images



Anti-5-hydroxy Methylcytosine Rabbit Monoclonal Antibody (Clone RM236) (µg/ml)

ELISA of single-stranded DNA using Anti-5-hydroxy Methylcytosine Rabbit Monoclonal Antibody (Clone RM236). The plate was coated with streptavidin and then biotinylated single-stranded unmethylated DNA, 5-methylcytosine (5-mC) DNA, and 5-hydroxy methylcytosine (5-hmC) DNA. A serial dilution of RM236 was used as the primary antibody, and an alkaline phosphatase conjugated anti-rabbit IgG as the secondary antibody.



hMeDIP was performed using anti-5-hmC antibody (RM236) at a 10:1 DNA:Ab ratio. 1 ng of unmethylated, 5-methylcytosine (5-mC) or 5-hydroxy methylcytosine (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.

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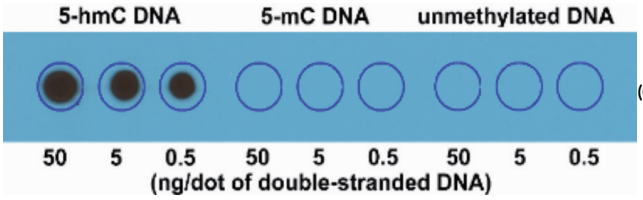
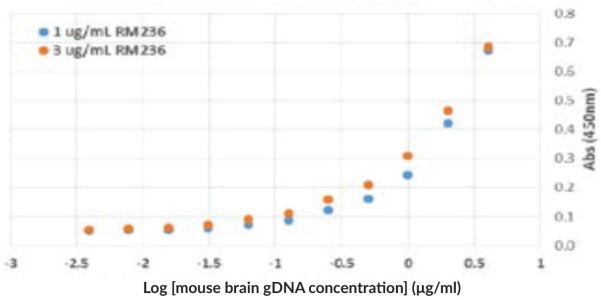
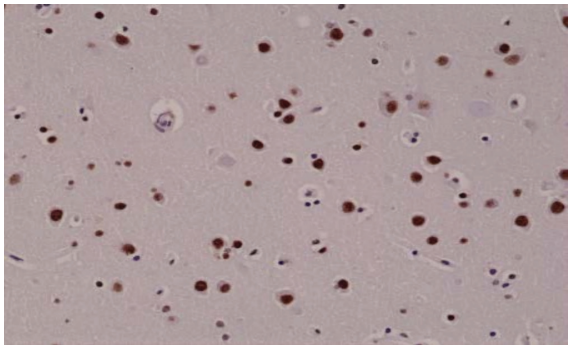


RM236
(0.2 µg/ml)

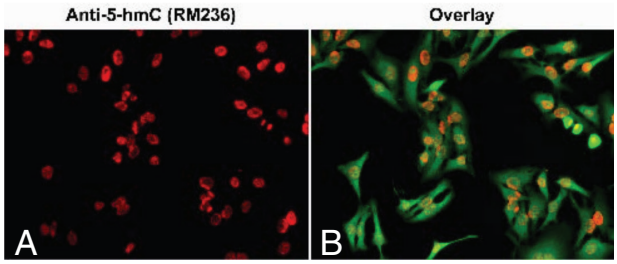
Dot blot of double-stranded DNA using Anti-5-hydroxy Methylcytosine Rabbit Monoclonal Antibody (Clone RM236) The membrane was pre-spotted with 50, 5, and 0.5 ng/dot of double stranded 5-hydroxy methylcytosine (5-hmC) DNA, 5-methylcytosine (5-mC) DNA, and unmethylated DNA.



Direct ELISA of mouse brain genomic DNA using anti-5-hmC antibody (RM236). The plate was directly coated with different concentrations of genomic DNA isolated from mouse brain tissue. 1 µg/ml or 3 µg/ml of RM236 was used as the primary antibody, and a HRP conjugated anti-rabbit IgG as the secondary antibody.



Immunohistochemical staining of formalin-fixed and paraffin-embedded human brain tissue sections, using Anti-5-hydroxy Methylcytosine Rabbit Monoclonal Antibody (Clone RM236).



Panel A: Immunocytochemical labeling of HeLa cells using 0.5 µg/ml Anti-5-hydroxy Methylcytosine Rabbit Monoclonal Antibody (Clone RM236) (red). *Panel B:* Actin filaments were 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 minutes at 37°C to denature the DNA.

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Description

5-Hydroxymethylcytosine (5-hmC) is an epigenetic modification formed from the oxidation of 5-methylcytosine by Tet dioxygenases.[1] 5-hmC is primarily a stable DNA modification, but it can be oxidized by Tet enzymes and its products further modified to generate nonmethylated cytosine, indicating a role as an intermediate in DNA demethylation as well.[2-4] It is associated with actively transcribed genes and recognized by a variety of proteins, including proteins involved in DNA repair and DNA metabolic processes.[2,3] 5-hmC has been found in all mammalian tissues but levels are higher in the brain relative to other tissues.[5] The percentage of genomic 5-hmC in mouse cerebellum increases during postnatal development until adulthood, and genes acquiring intragenic 5-hmC are enriched in pathways associated with age-related neurodegenerative disease pathways in adult mice.[6] In contrast, 5-hmC levels are reduced by up to 8-fold in cancer tissues.[1,7] Cayman's 5-Hydroxymethylcytosine Polyclonal Antibody can be used for dot blot, ELISA, hydroxymethylated DNA immunoprecipitation (hMeDIP), immunocytochemistry (ICC), and immunohistochemistry (IHC) applications.

Reference

1. Tahiliani, M., Koh, K.P., Shen, Y., *et al.* Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* **324(5929)**, 930-935 (2009).
2. Bachman, M., Uribe-Lewis, S., Yang, X., *et al.* 5-Hydroxymethylcytosine is a predominantly stable DNA modification. *Nat. Chem.* **6(12)**, 1049-1055 (2014).
3. Spruijt, C.G., Gnerlich, F., Smits, A.H., *et al.* Dynamic readers for 5-(hydroxy)methylcytosine and its oxidized derivatives. *Cell* **152(5)**, 1146-1159 (2013).
4. Breiling, A. and Lyko, F. Epigenetic regulatory functions of DNA modifications: 5-methylcytosine and beyond. *Epigenetics Chromatin* **8**, 24 (2015).
5. Globisch, D., Münzel, M., Müller, M., *et al.* Tissue distribution of 5-hydroxymethylcytosine and search for active demethylation intermediates. *PLoS One* **5(12)**, e15367 (2010).
6. Song, C.X., Szulwach, K.E., Fu, Y., *et al.* Selective chemical labeling reveals the genome-wide distribution of 5-hydroxymethylcytosine. *Nat. Biotechnol.* **29(1)**, 68-72 (2011).
7. Wang, Z., Du, M., Yuan, Q., *et al.* Epigenomic analysis of 5-hydroxymethylcytosine (5hmC) reveals novel DNA methylation markers for lung cancers. *Neoplasia* **22(3)**, 154-161 (2020).

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