

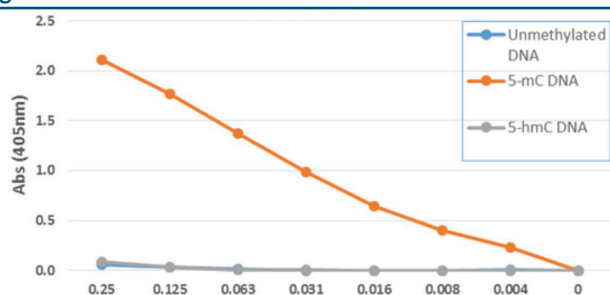
PRODUCT INFORMATION

Anti-5-methyl Cytosine Rabbit Monoclonal Antibody (Clone RM231) Item No. 20722

Overview and Properties

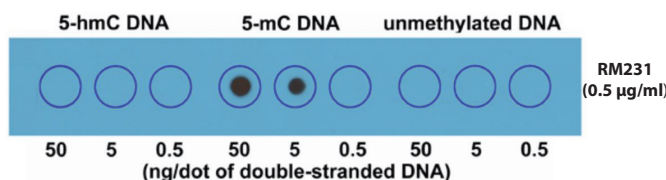
Contents:	This vial contains 50 µg of protein A-affinity purified antibody.
Synonyms:	5-mC
Immunogen:	BSA-conjugated 5-methyl cytosine
Cross Reactivity:	(+) 5-methyl cytosine in both single-stranded and double-stranded DNA; (-) Non-methylated cytosine, hydroxymethyl cytosine in DNA
Species Reactivity:	(+) All species
Form:	Liquid
Storage:	-20°C (as supplied)
Stability:	≥1 year
Storage Buffer:	PBS with 50% glycerol, 1% BSA, and 0.09% sodium azide
Concentration:	1.0 mg/ml
Clone:	RM231
Host:	Rabbit
Isotype:	IgG
Applications:	Dot blot (DB), ELISA, immunocytochemistry (ICC), immunohistochemistry (IHC), and methylated DNA immunoprecipitation (MeDIP); the recommended starting dilution for DB, ICC and FC is 0.5-2 µg/ml, 0.1-1 µg/ml for ELISA, and 0.2-2 µg/ml for MeDIP. Other applications were not tested, therefore optimal working concentration/dilution should be determined empirically.

Images



Anti-5-methyl Cytosine Rabbit Monoclonal Antibody (Clone RM231) (µg/ml)

ELISA of single stranded DNA using Anti-5-methyl Cytosine Rabbit Monoclonal Antibody (Clone RM231). The plate was coated with streptavidin and then biotinylated single stranded unmethylated DNA, 5-Methylcytosine (5-mC) DNA, and 5-Hydroxymethylcytosine (5-hmC) DNA. A serial dilution of Anti-5-methyl Cytosine Rabbit Monoclonal Antibody (Clone RM231) was used as the primary antibody, and an alkaline phosphatase conjugated anti-rabbit IgG as the secondary antibody.



Dot blot of double stranded DNA using Anti-5-methyl Cytosine Rabbit Monoclonal Antibody (Clone RM231). The 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 Anti-5-methyl Cytosine Rabbit Monoclonal Antibody (Clone RM231).

WARNING
THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

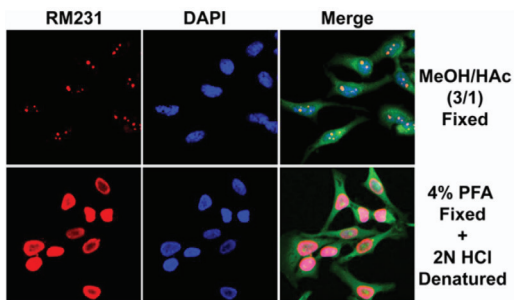
SAFETY DATA
This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent via email to your institution.

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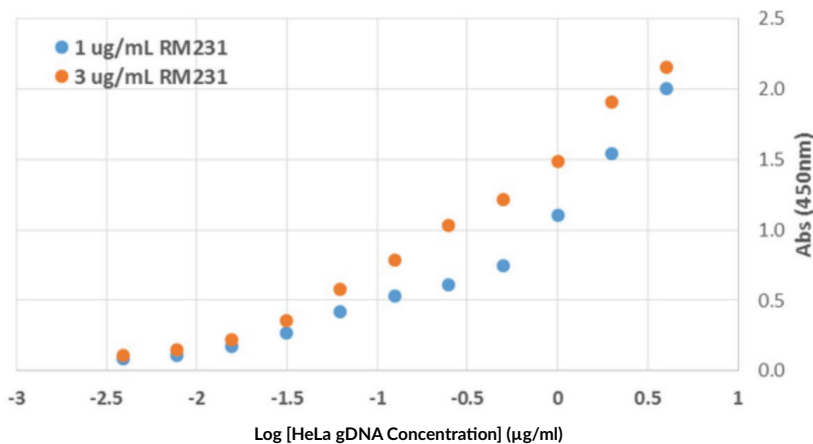
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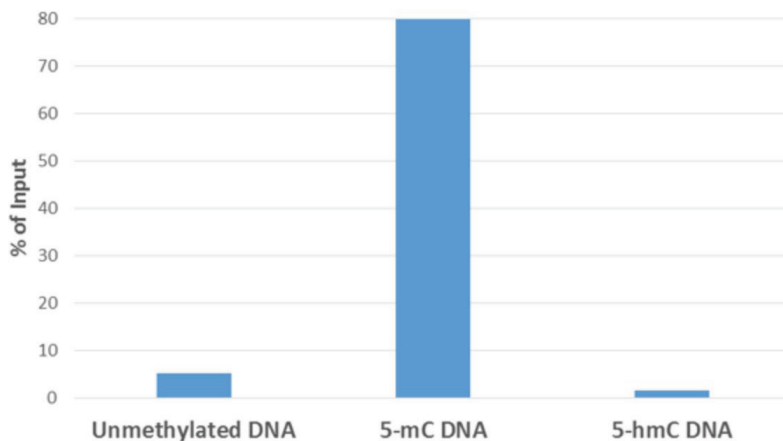
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Immunofluorescent labeling of HeLa cells using Anti-5-methyl Cytosine Rabbit Monoclonal Antibody (Clone RM231) (red). Actin filaments have been labeled with fluorescein phalloidin (green), and nuclei stained with DAPI (blue).



Direct ELISA of HeLa cell genomic DNA using Anti-5-methyl Cytosine Rabbit Monoclonal Antibody (Clone RM231). The plate was directly coated with different concentrations of genomic DNA isolated from HeLa cells. 1 or 3 µg/ml of Anti-5-methyl Cytosine Rabbit Monoclonal Antibody (Clone RM231) was used as the primary antibody, and a HRP-conjugated anti-rabbit IgG as the secondary antibody.



MeDIP was performed using Anti-5-methyl Cytosine Rabbit Monoclonal Antibody (Clone RM231) at a 2:1 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. Real time PCR was then performed to determine the capture of DNA standard as in % of input.

PRODUCT INFORMATION



Description

DNA methylation of cytosine to 5-methyl cytosine (5-mC) is an epigenetic mark that is relatively common in both plants and mammals and plays a role in transcriptional and chromatin regulation. In healthy cells, DNA methylation occurs at CpG sites, but CpG islands are generally not modified. However, tumor cells show an increase in CpG island methylation and a decrease in individual CpG sites.¹ This 'hypo-methylation' can lead to aberrant activation of genes. Anti-5-methyl Cytosine Rabbit Monoclonal Antibody (Clone RM231) is a probe for immunochemical detection of 5-methyl cytosine by methylated DNA immunoprecipitation, IHC, ICC, ELISA, or dot blot.

Reference

1. Huang, Y., Pastor, W. A., Zepeda-Martínez, J. A., *et al.* The anti-CMS technique for genome-wide mapping of 5-hydroxymethylcytosine. *Nature Protocols* **7(10)**, 1897-1908 (2012).

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