abcam

ab214727

Anti-5-methylcytosine (5-mC) antibody [RM231]

Anti-5-methylcytosine (5-mC) antibody [RM231] (ab214727) is a rabbit monoclonal antibody detecting 5-methylcytosine (5-mC) in **Flow Cytometry, IP, IHC-P, ELISA, Dot Blot**.

- Recombinant format for unrivalled batch-batch consistency
- Over 10 publications

Recombinant

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	Preservative: 0.09% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine), 1% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	Chemical / Small Molecule corresponding to 5-Methylcytosine.
Clone number	RM231
Purification technique	Affinity purification Protein A
Specificity	ab214727 reacts to 5-methylcytosine in both single-stranded and double-stranded DNA. No cross reactivity with non-methylated cytosine and hydroxymethylcytosine in DNA.
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC

Tested

Species	Modified Nucleic Acid
Dilution info	0.5-2 µg/mL
Notes	-

Flow Cyt

Tested

Species	Modified Nucleic Acid
Dilution info	0.5-2 µg/mL
Notes	-

ELISA

Tested

Species	Modified Nucleic Acid
Dilution info	0.1-3 µg/mL
Notes	-

Dot

Tested

Species	Modified Nucleic Acid
Dilution info	0.5-2 µg/mL
Notes	-

MeDIP

Tested

Species	Modified Nucleic Acid
Dilution info	0.2-2 µg/mL
Notes	-

IHC-P

Tested

Species	Modified Nucleic Acid
Dilution info	-
Notes	-

Target data

See full target information 5-Methylcytosine

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-5-methylcytosine (5-mC) antibody [RM231] (ab214727) is a rabbit recombinant monoclonal antibody and is validated for use in Flow Cytometry (Flow Cyt), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), ELISA, Dot Blot. **Trusted by the scientific community**
Anti-5-methylcytosine (5-mC) [RM231] (ab214727) was first used in a scientific publication in 2016 and has been cited over 10 times in peer-reviewed journals.

Other related products
We have a range of other formats of antibody clone [RM231] also available for your convenience: ab214727, ab319975, Carrier free - ab323581

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	5-methylcytosine often referred to as 5-mC 5-methyl cytosine or 5-methylcytidine is a methylated form of the nucleobase cytosine found in the DNA of various organisms. This modification typically occurs at the carbon 5 position of the cytosine ring contributing to the epigenetic regulation of gene expression. While the mass of 5-mC itself is relatively small since it is a modified nucleotide its impact on gene regulation is significant. 5-mC is widely expressed in the genome of higher eukaryotes particularly in CpG dinucleotides where it plays a role in mammalian development and cell differentiation.
Biological function summary	Cytosine methylation affects DNA-protein interactions leading to changes in chromatin structure and the regulation of gene activity. 5-methylcytosine is an important player in the chromatin modification complex where it collaborates with other epigenetic marks to control gene expression profiles. This modified nucleotide helps to stabilize gene silencing maintaining the closed chromatin state which inhibits the transcription machinery's access to specific genomic regions. Such silencing is vital during processes like X-chromosome inactivation and imprinting.
Pathways	Researchers have identified 5-mC within two critical pathways: DNA methylation and demethylation cycle and histone modification pathway. In the DNA methylation pathway 5-mC interacts closely with proteins like DNA methyltransferases (DNMTs) which directly add methyl groups to cytosine bases. In the context of histone modification 5-mC influences the binding of methyl-CpG-binding domain (MBD) proteins which read and interpret methylated DNA marks affecting histone modification and DNA accessibility.
Associated diseases and disorders	The dysregulation of 5-methylcytosine patterns associates prominently with cancer and neurological disorders. Abnormal methylation patterns where 5-mC is either hypermethylated or hypomethylated can lead to the silencing of tumor suppressor genes or activation of oncogenes forming a basis for cancer development. In neurological disorders altered 5-mC levels link to conditions such as Rett syndrome where the dysfunction of the associated protein MeCP2 an important reader of 5-mC contributes to the disease pathology.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

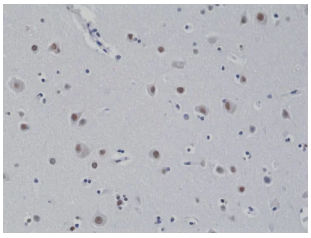
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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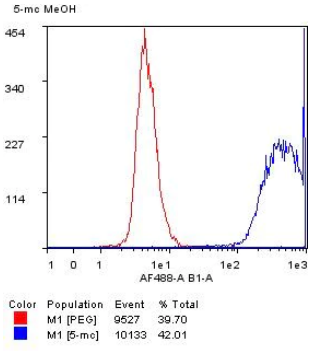
7 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-5-methylcytosine (5-mC) antibody [RM231] (ab214727)

5-methylcytosine (5-mC) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-5-methylcytosine (5-mC) antibody

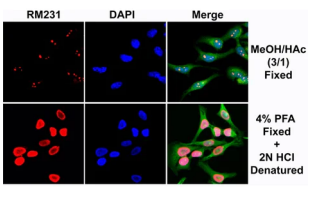
ab214727 staining 5-methylcytosine (5-mC) in human brain tissue sections by Immunohistochemistry (paraformaldehyde-fixed, paraffin-embedded sections). Tissue was blocked with 5% normal goat serum for 60 minutes at 22°C. Samples were incubated with primary antibody (20ng/ml) for 1 hour at 22°C. A HRP-conjugated Goat anti-rabbit IgG polyclonal was used as the secondary antibody.



Flow Cytometry - Anti-5-methylcytosine (5-mC) antibody [RM231] (ab214727)

5-methylcytosine (5-mC) Flow Cytometry staining using rabbit Anti-5-methylcytosine (5-mC) antibody

Flow Cytometry analysis of 5-mC expression in HEK293 cells using ab214727. The cells were fixed with ice-cold MeOH, permeabilized with 0.5% Triton X-100, denatured with 2N HCl, then stained with ab214727 (Blue) or with a negative control antibody (Red).



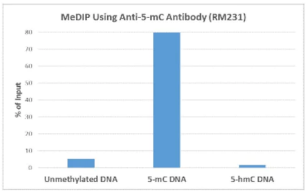
Immunocytochemistry - Anti-5-methylcytosine (5-mC) antibody [RM231] (ab214727)

5-methylcytosine (5-mC) Immunocytochemistry staining using rabbit Anti-5-methylcytosine (5-mC) antibody

Immunocytochemical analysis of HeLa cells using two different fixation/denaturation conditions and ab214727 at 2 µg/mL dilution (red); actin filaments have been labeled with fluorescein phalloidin (green), and nuclei stained with DAPI (blue).

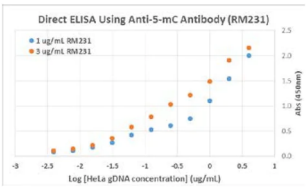
Chromatin denaturation is required to expose the epitopes in DNA and allow the antibody to efficiently detect 5mC. Stronger denaturing conditions such as HCl (bottom panels) will result in enhanced nuclear staining compared to weaker denaturing conditions such as acetic acid (HAc, top panels).

However, stronger denaturants such as using HCl may alter or degrade other molecules and intracellular structures, which can be problematic for experiments involving multi-color staining or looking at subcellular morphology. For those experiments we would suggest using weaker denaturants such as HAc.



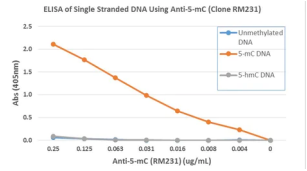
MeDIP - Anti-5-methylcytosine (5-mC) antibody [RM231] (ab214727)

MeDIP was performed using anti-5-mC antibody (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 1ug 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.



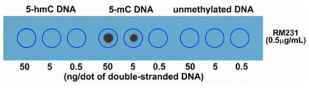
ELISA - Anti-5-methylcytosine (5-mC) antibody [RM231] (ab214727)

Direct ELISA of HeLa cell genomic DNA using anti-5-mC antibody (RM231). The plate was directly coated with different concentrations of genomic DNA isolated from HeLa cells. 1 ug/mL or 3 ug/mL of ab214727 was used as the primary antibody, and a HRP conjugated anti-rabbit IgG as the secondary antibody.



ELISA - Anti-5-methylcytosine (5-mC) antibody [RM231] (ab214727)

ELISA of single stranded DNA using ab214727 in a serial dilution. The plate was coated with streptavidin and then biotinylated single stranded unmethylated DNA, 5-Methylcytosine (5-mC) DNA, and 5-Hydroxymethylcytosine (5-hmC) DNA. Secondary antibody: alkaline phosphatase conjugated anti-rabbit IgG.



Dot Blot - Anti-5-methylcytosine (5-mC) antibody [RM231] (ab214727)

Dot blot of double stranded DNA using ab214727 at 0.5 ug/mL. 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 ab214727.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.