abcam

**ab214728**

# Anti-5-hydroxymethylcytosine (5-hmC) antibody [RM236]

Anti-5-hydroxymethylcytosine (5-hmC) antibody [RM236] (ab214728) is a rabbit monoclonal antibody detecting 5-hydroxymethylcytosine (5-hmC) in **Flow Cytometry, IP, IHC-P, ICC/IF, ELISA, Dot Blot**.

- Recombinant format for unrivalled batch-batch consistency
- Over 20 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-Hydroxymethylcytosine. |
| **Clone number** | RM236 |
| **Purification technique** | Affinity purification Protein A |
| **Specificity** | ab214728 reacts to 5-hydroxymethylcytosine in both single-stranded and double-stranded DNA. No cross reactivity with non-methylated cytosine and methylcytosine in DNA. |
| **Concentration** | 1 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

## Dot

**Tested**

| | |
|---|---|
| **Species** | Modified Nucleic Acid |
| **Dilution info** | 0.2-1 g/mL |
| **Notes** | - |

## IHC-P

**Tested**

| | |
|---|---|
| **Species** | Modified Nucleic Acid |
| **Dilution info** | 0.1-1 µg/mL |
| **Notes** | - |

## ICC/IF

**Tested**

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

## ELISA

**Tested**

| | |
|---|---|
| **Species** | Modified Nucleic Acid |
| **Dilution info** | 0.1-1 µg/mL |
| **Notes** | - |

## MeDIP

**Tested**

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

| | |
|---|---|
| **Notes** | - |

## Flow Cyt

### Tested

| | |
|---|---|
| **Species** | Modified Nucleic Acid |
| **Dilution info** | 1-5 µg/mL |
| **Notes** | - |

## Target data

See full target information 5-hmC)

## 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-hydroxymethylcytosine (5-hmC) antibody [RM236] (ab214728) is a rabbit recombinant monoclonal antibody and is validated for use in Flow Cytometry (Flow Cyt), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF), ELISA, Dot Blot.

**Trusted by the scientific community**
Anti-5-hydroxymethylcytosine (5-hmC) [RM236] (ab214728) was first used in a scientific publication in 2016 and has been cited over 20 times in peer-reviewed journals.

**Other related products**

We have a range of other formats of antibody clone [RM236] also available for your convenience: ab214728, ab319976, Carrier free - ab323582

## Supplementary info

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

| | |
|---|---|
| **Activity summary** | 5-hydroxymethylcytosine (5-hmC) also known as hydroxymethylcytosine represents a significant epigenetic modification in DNA. This target with a mass of approximately 63.3 emerges through DNA demethylation processes where ten-eleven translocation (TET) enzymes oxidize 5-methylcytosine (5-mC). It is found largely in the brain where it shows high levels of expression but it can also appear in other tissues to a lesser extent. The modification is important in regulating gene expression and maintaining cellular identity. |
| **Biological function summary** | 5-hydroxymethylcytosine plays a critical role in epigenetic regulation and serves as an intermediary in the active DNA demethylation process. It is not part of a stable complex but interacts transiently during epigenetic reconfiguration. The presence of 5-hmC marks regulatory regions of genes and is involved in transcriptional activation or repression depending on the cellular context. Its levels are dynamic influenced by various developmental stages and environmental conditions. |
| **Pathways** | This DNA modification is an important player in pathways related to cellular differentiation and development. It is integrally involved in the DNA demethylation pathway where TET enzymes mediate its formation from 5-methylcytosine. This pathway also involves other modifications leading to cytosine turnover linking 5-hmC to broader chromatin remodeling processes. Proteins interacting with 5-hmC include TET enzymes and other DNA maintenance proteins which highlight its role in maintaining genomic stability. |
| **Associated diseases and disorders** | Aberrant 5-hmC levels show connection to several pathological states including cancer and neurological disorders. Altered hydroxymethylation patterns often associate with tumorigenesis particularly in gliomas where the misregulation of 5-hmC accompanies cancer progression. Neurological disorders like Rett syndrome also exhibit altered levels of 5-hmC implicating its role in neurodevelopmental processes. In these diseases mutations in proteins such as TET enzymes link to disrupted 5-hmC levels indicating its critical involvement in both oncogenesis and neurodevelopmental regulation. |

## 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.

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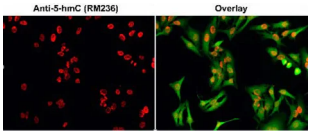
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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

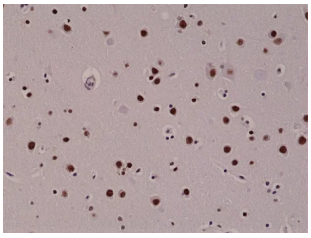



### Immunocytochemistry/ Immunofluorescence - Anti-5-hydroxymethylcytosine (5-hmC) antibody [RM236] (ab214728)

5-hydroxymethylcytosine (5-hmC) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-5-hydroxymethylcytosine (5-hmC) antibody

Immunocytochemical staining of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells using 0.5 µg/mL ab214728 (red). Actin filaments was 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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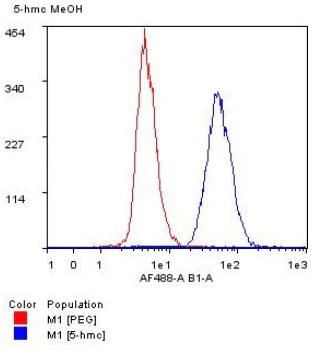


### Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-5-hydroxymethylcytosine (5-hmC) antibody [RM236] (ab214728)

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

Immunohistochemical analysis of paraffin-embedded human brain tissue labeling 5-hydroxymethylcytosine (5-hmC) with ab214728 at 1 µg/mL.

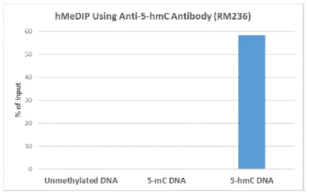
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## Flow Cytometry - Anti-5-hydroxymethylcytosine (5-hmC) antibody [RM236] (ab214728)

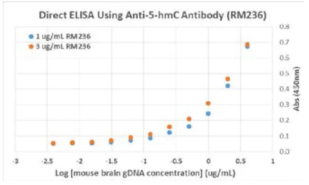
5-hydroxymethylcytosine (5-hmC) Flow Cytometry staining using rabbit Anti-5-hydroxymethylcytosine (5-hmC) antibody

Flow Cytometry analysis of 5-hmC expression in HEK293 cells using ab214728. The cells were fixed with ice-cold MeOH, permeabilized with 0.5% Triton X-100, denatured with 2N HCl, then stained with ab214728 (Blue) or with a negative control antibody (Red).



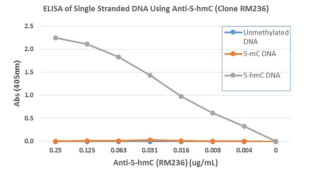

## MeDIP - Anti-5-hydroxymethylcytosine (5-hmC) antibody [RM236] (ab214728)

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-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.



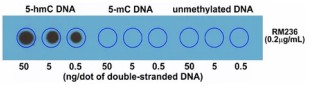

## ELISA - Anti-5-hydroxymethylcytosine (5-hmC) antibody [RM236] (ab214728)

Direct ELISA of mouse brain genomic DNA using ab214728. The plate was directly coated with different concentrations of genomic DNA isolated from mouse brain tissue. 1 ug/mL or 3 ug/mL of ab214728 was used as the primary antibody and an HRP conjugated anti-rabbit IgG as the secondary antibody.




## ELISA - Anti-5-hydroxymethylcytosine (5-hmC) antibody [RM236] (ab214728)

ELISA of single stranded DNA using ab214728 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-hydroxymethylcytosine (5-hmC) antibody [RM236] (ab214728)

Dot blot of double stranded DNA using ab214728 at 0.2 µg/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 ab214728.

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