

## Monoclonal Antibody to 5-Hydroxymethylcytosine - Purified

|                                      |  |
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| <b>Alternate names:</b>              | 5' hydroxymethyl cytosine, 5'OH methyl cytosine, 5'OHmeC, 5-hmec   |
| <b>Catalog No.:</b>                  | AM33116PU-N  |
| <b>Quantity:</b>                     | 0.1 mg   |
| <b>Concentration:</b>                | 1.0 mg/ml  |
| <b>Background:</b>                   | <p>5-Hydroxymethylcytosine is a DNA pyrimidine nitrogen base formed from the enzymatic conversion of 5-methylcytosine into 5-hydroxymethylcytosine by the TET family of iron-dependent oxygenases. Data suggests that every mammalian cell contains 5-hydroxymethylcytosine, but the levels vary depending on the cell type; data also suggests that levels of 5-hydroxymethylcytosine increases with age. The highest levels are found in neuronal cells of the central nervous system and certain mammalian tissues such as mouse Purkinje and granule neurons. Although the exact function has not been fully elucidated, studies suggest that 5-hydroxymethylcytosine may regulate gene expression or initiate DNA demethylation. 5-hydroxymethylcytosine (5-hmC) is a modified base form of cytosine recently found in human/mouse brain and in embryonic stem cells. This DNA pyrimidine nitrogen base can be generated by oxidation of 5-methylcytosine, a reaction mediated by the ten-eleven translocation (TET) family of the 5-mC hydroxylases. The function of this base is still not elucidated but it is believed to play an important role in switching genes on and off.</p> |
| <b>Host / Isotype:</b>               | Mouse / IgG1   |
| <b>Recommended Isotype Controls:</b> | AM03095PU-N  |
| <b>Clone:</b>                        | 4D9  |
| <b>Immunogen:</b>                    | Modified 5-Hydroxymethylcytosine found in DNA vertebrates.   |
| <b>Format:</b>                       | <b>State:</b> Liquid purified IgG fraction<br><b>Purification:</b> Affinity Chromatography on Protein A<br><b>Buffer System:</b> 10mM PBS, 0.15M Sodium Chloride, pH 7.4<br><b>Preservatives:</b> 0.01 % Thimerosal  |
| <b>Applications:</b>                 | <b>ELISA:</b> 1/1000 (See <i>Figure.1</i> )<br><b>Dot Blot:</b> 1/2000 (See <i>Table.1</i> )<br><b>hMeDIP:</b> 1-3 µg per Immunoprecipitaion.<br><b>Immunofluorescence:</b> 1/500.<br>Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.   |
| <b>Specificity:</b>                  | This antibody detects 5-Hydroxymethylcytosine (5-hmC).<br>Expected to detect 5-Hydroxymethylcytosine in all Species.   |

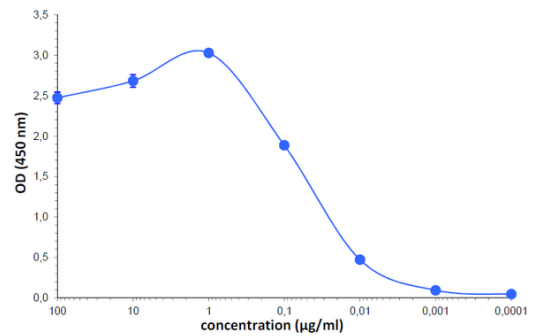
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Material Safety Datasheets are available at [www.acris-antibodies.com](http://www.acris-antibodies.com) or on request.

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**Storage:** Upon receipt, store (in aliquots) at -20°C to -80°C.  
Avoid repeated freezing and thawing.  
Shelf life: one year from despatch.

**General Readings:** 1. Li W, Liu M. Distribution of 5-hydroxymethylcytosine in different human tissues. *J Nucleic Acids*. 2011;2011:870726. doi: 10.4061/2011/870726. Epub 2011 Jun 9. PubMed PMID: 21772996.  
2. Guo JU, Su Y, Zhong C, Ming GL, Song H. Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. *Cell*. 2011 Apr 29;145(3):423-34. doi: 10.1016/j.cell.2011.03.022. Epub 2011 Apr 14. PubMed PMID: 21496894.

**Pictures:** **Figure 1. Determination of the 5-hmC monoclonal antibody titer.** Direct ELISA performed with serial dilutions of the 5-hmC monoclonal antibody (4D9) against 5-hmC in antigen coated wells. Antigen used: BSA coupled to 5-hmC base. Estimated titer: 0.05 µg/ml.



**Table 1. Dot Blot analysis of the 5hmC monoclonal antibody (4D9) with DNA standard containing Cytosine (C), 5-methylcytosine (5-mC) or 5-hydroxymethylcytosine (5-hmC).** 10ng of C, 5-mC and 5-hmC of the “5-Methylcytosine & 5-Hydroxymethylcytosine DNA Standard Set” (D5405, Zymo Research) were spotted on a membrane (Amersham Hybond ECL). The membrane was incubated with 0.5 µg/ml of the 5-hmC monoclonal antibody (dilution 1/2,000) then with peroxidase conjugated Goat anti-Mouse IgG (dilution 1/3000). Specific signal was only observed with 5-hmc DNA standard.

| C | 5-mC | 5-hmC | TBS |
|---|------|-------|-----|
|   |      |       |     |