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## TECHNICAL DATASHEET

## Monoclonal Anti-5-Hydroxymethylcytosine

### Clone 4D9

Ref # BIO.020.3

### Product description

Description	Monoclonal antibody to 5-Hydroxymethylcytosine (5-hmC)
Host species	Mouse
Validated applications	hMeDIP, ELISA, Dot Blot (other applications not tested)
Species reactivity	All species
Specificity	5-hmC
Epitope	Modified 5-hydroxymethylcytosine found in DNA vertebrates

### Target exploration

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.

### Properties

Form	Liquid or lyophilized
Storage instructions	Store aliquoted at -20°C or -80°C. Avoid repeated freeze / thaw cycles
Storage buffer	Phosphate Buffered Saline 10 mM – NaCl 0.15 M– pH 7.4
Purity	Purified IgG fractions prepared by affinity chromatography on Protein A (> 95%)
Isotype	IgG1
Concentration	1 mg/ml
Restrictions	For research use only

# Applications





**hMeDIP:** 1-3µg per IP (Suggested quantity)\*

**IF:** 1:500 (Suggested dilution)\*

**Dot Blotting:** 1:2,000 (Suggested dilution, see table 1)\*

**ELISA:** 1:1,000 (Suggested dilution, see fig.1)\*

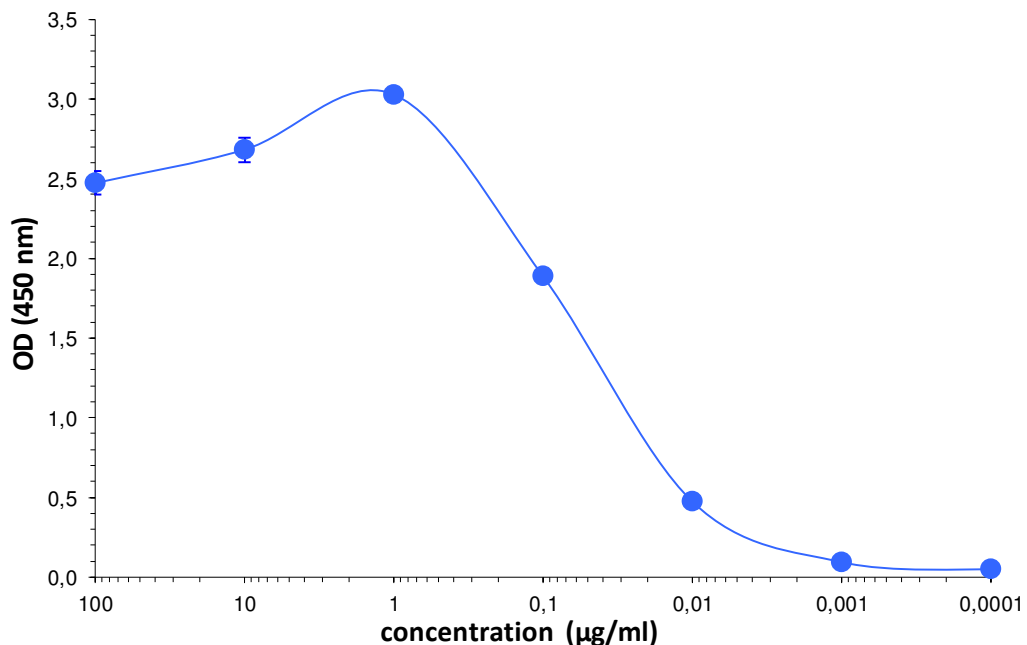
\*Optimal quantity/dilution should be determined by the end user

C	5-mC	5-hmC	TBS
			

**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) (115-036-044, Immunotech). Specific signal was only observed with 5-hmc DNA standard.



**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: 0.05µg/ml.

## Associated products

Monoclonal antibody to 5-methylcytosine (5-mC) clone 33D3, catalogue number: BIO.000.1

## Publications

Li, W., and Liu, M. (2011). Distribution of 5-hydroxymethylcytosine in different human tissues. *J Nucleic Acids* 2011, 870726.  
Guo, Junjie U.; Su, Yijing, Zhong, Chun, Ming, Guo-li, Song, Hongjun (2011). Hydroxylation of 5-Methylcytosine by TET1 Promotes Active DNA Demethylation in the Adult Brain". *Cell* 145 (3): 423–434