

Anti-5-Hydroxymethylcytosine Polyclonal Antibody



ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

Cat. Nos. A4001-50 (50 µg in 50 µl volume)

A4001-200 (200 µg in 200 µl volume)

Storage: -20 °C for normal use, or -80 °C for long-term storage

Product Information

Description:

Recent advances in the field of epigenetics have identified 5-hydroxymethylcytosine (5-hmC) as a key factor in the regulation of gene expression, with substantial implications in the study of tissue differentiation, neurological development, and carcinogenesis. Studies of this epigenetic marker are typically confounded by a lack of reliable methodology for differentiation from the highly prevalent 5-methylcytosine in a DNA sample.

The rabbit Anti-5-hydroxymethylcytosine polyclonal antibody has been developed in order to robustly distinguish between hydroxymethylated DNA and methylated or unmodified DNA. Specificity of the antibody is enhanced such that crossreactivity with unmodified and methylated templates is suppressed to near-background levels. The antibody has been extensively tested and validated in ELISA and immunoprecipitation-based enrichment assays, and is suitable for use in further applications including immunohistochemical labeling and chromatographic blotting.

Product Type: Polyclonal Antibody

Format: Purified from serum and dialyzed.

Source: Rabbit

Isotype: IgG1

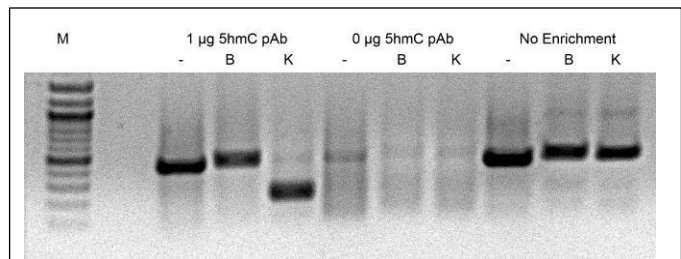
Approximate Peptide Concentration: 1 mg/ml per BCA quantitation.

Specificity: 5-hydroxymethylcytosine in single-stranded DNA from any mammalian, plant, insect, or microbial sources, as well as artificial templates used for standardization.

Buffer Solution: Antibody is provided in PBS at pH 7.5 with sodium azide added to 0.05% as a preservative.

Storage: Store at -20 °C with prolonged storage at -80 °C. Avoid repeated freeze/thawing.

Stability/Shelf Life: With proper storage, the antibody is stable for one year from the date of receipt.



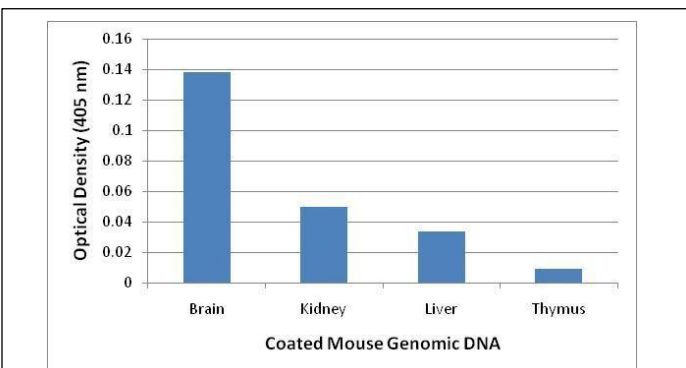
Hydroxymethylated DNA is efficiently enriched using the anti-5-hydroxymethylcytosine antibody. DNA was immunoprecipitated from 1 ng of a mixed non-methylated/methylated/hydroxymethylated (10:1:1) DNA population. This population was comprised of a mixture of non-methylated plasmid DNA, a methylated version of the same plasmid containing a point mutation that introduces a BamHI restriction site, and a hydroxymethylated version of the same plasmid with a KpnI restriction site. After IP, the region of DNA containing the restriction site was amplified by PCR, digested with either BamHI (B) or KpnI (K), and visualized in a 1.4% (w/v) agarose/TAE/EtBr gel. The results indicate high sensitivity of the antibody for 5hmC DNA with no detectable crossreactivity to 5mC DNA.

Application and Usage Information

Application	Yes	Recommended Dilution
ELISA	Yes	1:1,000 to 1:10,000
Immunoblotting	Yes	Varies
Immunofluorescence	Yes	Varies
Immunohistology	Yes	Varies
Immunoprecipitation of Hydroxymethylated DNA	Yes	1 to 2 µg per IP

Also Available for 5-Hydroxymethylcytosine Analysis:

Product Name	Size	Catalog number
Mouse 5-mC and 5-hmC DNA Set	1 set	D5019
5-mC and 5-hmC DNA Standard Set	1 set	D5405
Quest 5-hmC Detection Kit™ (includes MspI)	25 preps 50 preps	D5410 D5411
Quest 5-hmC Detection Kit™ - Lite	25 preps 50 preps	D5415 D5416
CleanCapture™ 5-hmC Enrichment Kit	25 preps 50 preps	D5420 D5421
Quest 5-hmC ELISA Kit™	1 plate 2 plates	D5425 D5426
JBP1 (J-Binding Protein)	100 units 200 units	E2060 E2061
5-hmC Glucosyltransferase	100 units 200 units	E2026 E2027
Quest Taq™ Premix	50 rxns 200 rxns	E2050 E2051
Quest Taq™ qPCR Premix	50 rxns 200 rxns	E2052 E2053
DNA Degradase™	500 units 2000 units	E2016 E2017
DNA Degradase Plus™	500 units 2000 units	E2020 E2021



The anti-5-hydroxymethylcytosine polyclonal antibody demonstrates high sensitivity to 5-hmC in genomic DNA. In a standard ELISA workflow, 100 ng of purified genomic DNA from several murine tissue sources — brain, kidney, liver, and thymus — are coated per well. The anti-5-hydroxymethylcytosine antibody and the secondary antibody (anti-rabbit horseradish peroxidase conjugate) are used at 1:1,000 dilution in modified TBS and incubated at 37 °C for 1 hour. Optical density was measured after 1 hour of ABTS substrate incubation at room temperature. Independent quantitation of 5-hmC levels using LC/MS analysis of gDNA from the same tissue sources indicates brain at 0.548 %, kidney at 0.225%, liver at 0.107 %, and thymus at 0.030% (refer to Catalog #D5019), demonstrating high correlation with the colorimetric ELISA data above.

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Additional Tools for Epigenetics and 5-Methylcytosine Analysis:

Human HCT116 DKO Methylation Standards	1 set	D5014
Human HCT116 DKO Non-methylated DNA Standard	5 µg	D5014-1
Human HCT116 DKO Methylated DNA Standard	5 µg	D5014-2
Bisulfite Converted Universal Methylated Human DNA Standard	1 set	D5015
EZ DNA Methylation-Startup Kit	50 rxns	D5024
EZ DNA Methylation Kit	50 rxns 200 rxns	D5001 D5002
EZ DNA Methylation-Gold Kit	50 rxns 200 rxns	D5005 D5006
EZ DNA Methylation-Direct Kit	50 rxns 200 rxns	D5020 D5021
<i>E. coli</i> Non-methylated Genomic DNA	5 µg	D5016
Methylated-DNA IP Kit	10 preps	D5101
ChIP DNA Clean & Concentrator™	50 preps 50 preps	D5201 D5205
Zymo <i>Taq</i> ™ DNA Polymerase	50 rxns 200 rxns	E2001 E2002
Zymo <i>Taq</i> ™ PreMix (2X concentrated)	50 rxns 200 rxns	E2003 E2004
CpG Methylase (M.SssI)	200 units 400 units	E2010 E2011
GpC Methylase (M.CviPI)	200 units 1000 units	E2014 E2015
Anti-5-Methylcytosine Monoclonal Antibody	50 µg 200 µg	A3001-50 A3001-200

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This product is for research use only and should only be used by trained professionals. Wear protective gloves and eye protection. Follow the safety guidelines and rules of your research institution or facility.

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