

# 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

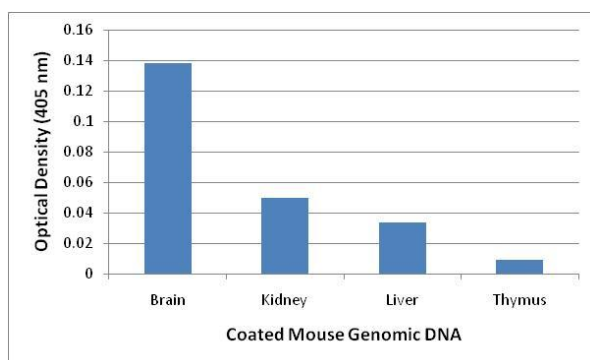
**Approximate Peptide Concentration:** 1 mg/ml per BCA quantitation.

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

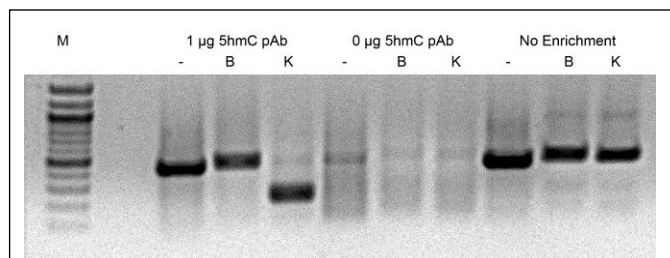
**Buffer Solution:** Antibody is provided in PBS at pH 7.5 with sodium azide added to 0.05% as a preservative.

**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.



**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.



**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<br>50 preps    | D5410<br>D5411 |
| Quest 5-hmC Detection Kit™ - Lite          | 25 preps<br>50 preps    | D5415<br>D5416 |
| CleanCapture™ 5-hmC Enrichment Kit         | 25 preps<br>50 preps    | D5420<br>D5421 |
| Quest 5-hmC ELISA Kit™                     | 1 plate<br>2 plates     | D5425<br>D5426 |
| JBP1 (J-Binding Protein)                   | 100 units<br>200 units  | E2060<br>E2061 |
| 5-hmC Glucosyltransferase                  | 100 units<br>200 units  | E2026<br>E2027 |
| QuestTaq™ Premix                           | 50 rxns<br>200 rxns     | E2050<br>E2051 |
| QuestTaq™ qPCR Premix                      | 50 rxns<br>200 rxns     | E2052<br>E2053 |
| DNA Degradase™                             | 500 units<br>2000 units | E2016<br>E2017 |
| DNA Degradase Plus™                        | 500 units<br>2000 units | E2020<br>E2021 |

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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<br>200 rxns     | D5001<br>D5002        |
| EZ DNA Methylation-Gold Kit                                 | 50 rxns<br>200 rxns     | D5005<br>D5006        |
| EZ DNA Methylation-Direct Kit                               | 50 rxns<br>200 rxns     | D5020<br>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<br>50 preps    | D5201<br>D5205        |
| ZymoTaq™ DNA Polymerase                                     | 50 rxns<br>200 rxns     | E2001<br>E2002        |
| ZymoTaq™ PreMix (2X concentrated)                           | 50 rxns<br>200 rxns     | E2003<br>E2004        |
| CpG Methylase (M.SssI)                                      | 200 units<br>400 units  | E2010<br>E2011        |
| GpC Methylase (M.CviPI)                                     | 200 units<br>1000 units | E2014<br>E2015        |
| Anti-5-Methylcytosine Monoclonal Antibody                   | 50 µg<br>200 µg         | A3001-50<br>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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