5-Hydroxymethylcytosine polyclonal antibody

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 5-hydroxymethylcytosine pAb 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. Overall, the 5-hydroxymethylcytosine pAb has low cross reactivity with cytosine and 5-methylcytosine versus other available antibodies; can be used with a variety of genomic DNA sources; and has high sensitivity to low masses of 5-hydroxymethylcytosine DNA.

This antibody is covered by our Worry-Free Guarantee.

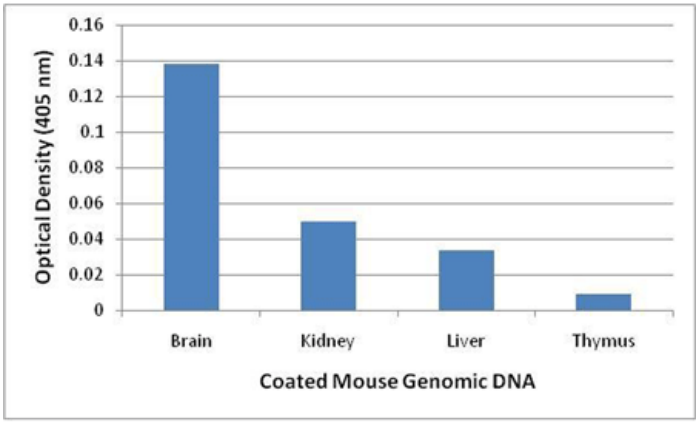
Ordering Information

Order Online »

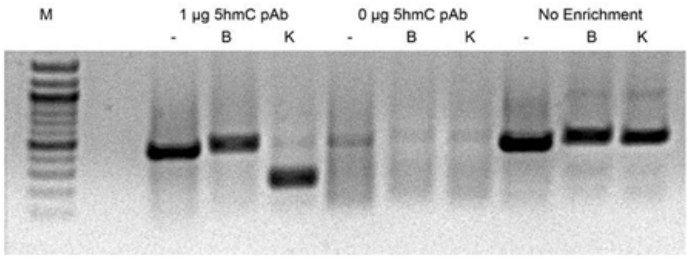
ADI-905-904-0050	50µg
ADI-905-904-0200	200µg

Manuals, SDS & CofA

View Online »



ELISA analysis: The 5-Hydroxymethylcytosine pAb demonstrates high sensitivity to 5-hydroxymethylcytosine 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 5-Hydroxymethylcytosine pAb and a secondary antibody (anti-rabbit horseradish peroxidase conjugate) are used at 1:1000 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-hydroxymethylcytosine levels using LC/MS analysis of genomic DNA from the same tissue sources indicates brain at 0.548%, kidney at 0.225%, liver at 0.107 %, and thymus at 0.030%, demonstrating high correlation with the colorimetric ELISA data.



Immunoprecipitation analysis: Hydroxymethylated DNA is efficiently enriched using the 5-Hydroxymethylcytosine pAb. 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 5-hydroxymethylcytosine pAb for 5-hydroxymethylcytosine DNA with no detectable crossreactivity to 5-methylcytosine DNA.

Handling & Storage

Long Term Storage	-20°C
Shipping	Blue Ice

Regulatory Status

RUO - Research Use Only

Product Details

Alternative Name	5-hmC
Application	ELISA, Immunoblot, IP
Formulation	Liquid. In PBS, pH 7.5, containing 0.05% sodium azide.
Host	Rabbit
Immunogen	5-Hydroxymethylcytosine
Isotype	IgG1
Purity Detail	Purified.
Recommendation Dilutions/Conditions	ELISA (1:1,000-5,000)Immunoblotting (Dot blotting, 1:500-2,000)Immunoprecipitation (1-2 µg).Suggested dilutions/conditions may not be available for all applications.Optimal conditions must be determined individually for each application.
Species Reactivity	Species independent
Specificity	Recognizes 5-hydroxymethylcytosine in single-stranded DNA from any mammalian, plant, insect, or microbial sources, as well as artificial templates used for standardization.
Worry-free Guarantee	**This antibody is covered by our Worry-Free Guarantee .**

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