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Datasheet for 200-301-V06

5-hmc Antibody

Overview

Description:	Anti-5-hydroxymethylcytosine (MOUSE) Monoclonal Antibody - 200-301-V06
Item No.:	200-301-V06
Size:	50 µg
Applications:	Dot Blot, ELISA
Reactivity:	Human, Mouse, Broad
Host Species:	Mouse

Product Details

Background:	5-hydroxymethylcytosine (5-hmC) has been recently discovered in mammalian DNA. This results from the enzymatic conversion of 5-methylcytosine into 5-hydroxymethylcytosine by the TET family of oxygenases. So far, the 5-hmC bases have been identified in Purkinje neurons, in granule cells and embryonic stem cells where they are present at high levels (up to 0,6% of total nucleotides in Purkinje cells). Preliminary results indicate that 5-hmC may have important roles distinct from 5-mC. Although its precise role has still to be shown, early evidence suggests a few putative mechanisms that could have big implications in epigenetics: 5-hydroxymethylcytosine may well represent a new pathway to demethylate DNA involving a repair mechanism converting 5-hmC to cytosine and, as such open up entirely new perspectives in epigenetic studies. Due to the structural similarity between 5-mC and 5-hmC, these bases are experimentally almost indistinguishable. Recent articles demonstrated that the most common approaches (e.g. enzymatic approaches, bisulfite sequencing) do not account for 5-hmC. The development of the affinity-based technologies appears to be the most powerful way to differentially and specifically enrich 5-mC and 5-hmC sequences. Anti-5hmC Antibody is ideal for research in Epigenetics and Gene Expression.
Synonyms:	5-hydroxymethylcytosine
Host Species:	Mouse
Clonality:	Monoclonal
Format:	IgG1

Target Details

Reactivity:	Human, Mouse, Broad

Immunogen Type:	Other
Immunogen:	Anti-5hmC Antibody was produced in mice by repeated immunization with 5-hydroxymethlcytosine.
Purity/Specificity:	Anti-5hmC Antibody was purified by Protein A chromatography. Cross-reactivity with 5hmC from other sources has not been determined.

Application Details

Tested Applications:	Dot Blot, ELISA
Application Note:	Anti-5hmC Antibody is tested for Dot Blotting, ELISA, and Hydroxymethylated DNA Immunoprecipitation Sequencing. Specific conditions for reactivity should be optimized by the end user.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:500
IF:	User Optimized
Other:	hMeDIP: 2.5µg per IP. Dot Blot: 2 µg/ml

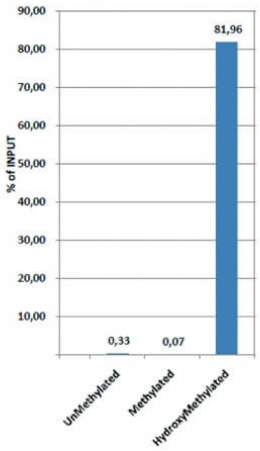
Formulation

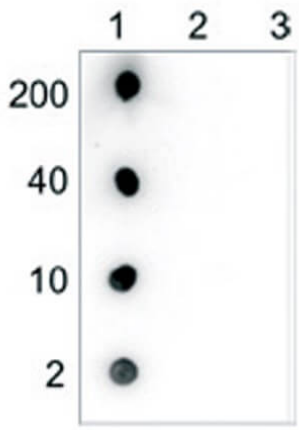
Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/ml by UV absorbance at 280 nm
Buffer:	0.01 M Sodium Phosphate, 0.25 M Sodium Chloride, pH 7.2
Preservative:	0.05% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images





ELISA
Mouse Anti-5-hydroxymethylcytosine Antibody immunoprecipitation was performed using the mouse monoclonal antibody directed against 5-hydroxymethylcytosine. The IgG isotype antibodies from mouse was used as negative control. The DNA was prepared and sonicated to have DNA fragments of 300-500 bp. 1 µg of human Hela cells DNA were spiked with non-methylated, methylated, and hydroxymethylated PCR fragments. The IP'd material has been analyzed by qPCR using the primer pair specific for the 3 different control sequences. The obtained results show that the mouse monoclonal for 5-hmC is highly specific for this base modification (no IP with non-methylated or methylated C bases containing fragments).

Dot Blot
Mouse Anti-5-hydroxymethylcytosine Antibody Dot Blot analysis. Lane 1: 5-hmC PCR controls. Lane 2: 5-mC PCR controls. Lane 3: cytosine PCR controls. Dilutions: 200ng, 40ng, 10ng, 2ng. Primary Antibody: mouse 5-hydroxymethylcytosine monoclonal antibody 2µg/mL (dilution 1:500). Secondary antibody: Dylight™488 rabbit secondary antibody at 1:10,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C. The membranes were exposed for 30 seconds.

Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.