

Datasheet for 200-401-X50**5-caC Antibody****Overview**

Description:	Anti-5-Carboxylcytosine (RABBIT) Antibody - 200-401-X50
Item No.:	200-401-X50
Size:	100 µg
Applications:	Dot Blot, IF, IP, Multiplex
Reactivity:	Human, Mouse, Broad
Host Species:	Rabbit

Product Details**Background:**

Until recently, 5-methylcytosine (5-mC) was the only known modification of DNA for epigenetic regulation. In 2009, however, a second methylated cytosine, 5-hydroxymethylcytosine (5-hmC) was discovered. This new modified base (also called the Sixth base) is generated by enzymatic conversion of 5-mC into 5-hmC by the TET family of oxygenases. Recent results indicate that 5-hmC plays important roles distinct from 5-mC. Although its precise role has still to be shown, early evidence suggests that 5-hmC may well represent a new pathway to demethylate DNA involving a repair mechanism converting 5-hmC to cytosine. This pathway could involve further oxidation of the hydroxymethyl group to a formyl or carboxyl group followed by either deformylation or decarboxylation. The carboxyl and formyl groups of 5-Formylcytosine (5-fC) and 5-Carboxylcytosine (5-caC) could be enzymatically removed without excision of the base. Due to their structural similarity, the different modified cytosine analogues are difficult to discriminate. The development of highly specific affinity-based reagents, such as antibodies, appears to be the most powerful way to differentially and specifically enrich 5-mC and 5-hmC sequences. Anti-5caC Antibody is ideal for research in Genetics and Epigenetics.

Synonyms:	5ca-CMP monophosphate
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Reactivity:	Human, Mouse, Broad
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Immunogen Type:	Other
Immunogen:	Anti-5caC Antibody was produced in rabbits by repeated immunizations with 5-carboxylcytosine (5caC).
Purity/Specificity:	Anti-5caC Antibody was purified by Protein A purification. Cross reactivity with other species was not tested but a wide range is presumed.

Application Details

Tested Applications:	Dot Blot, IF, IP, Multiplex
Application Note:	Anti-5caC Antibody is tested for Dot Blots, Immunofluorescence and Immunoprecipitation. 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.

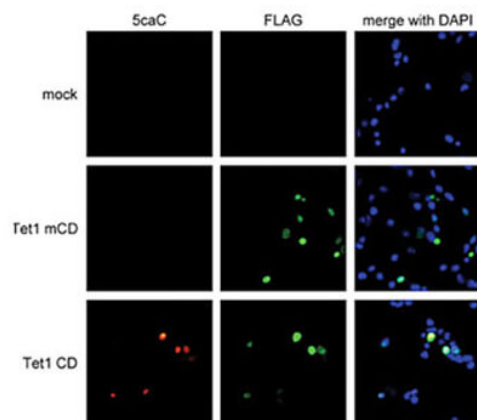
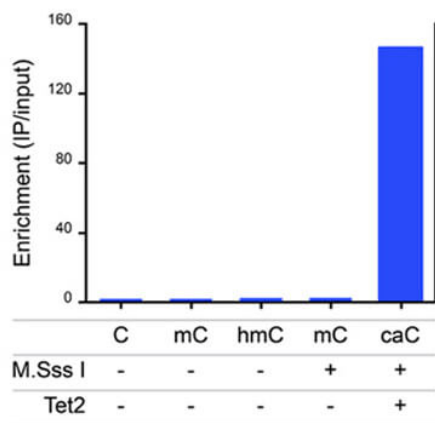
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1 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:	30% Glycerol

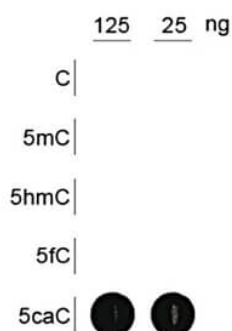
Shipping & Handling

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



anti-5caC (Protein A purified)



Immunoprecipitation

IP performed on 2 µg of J1 ES genomic DNA, spiked with 1 pg of a control DNA fragment (approximately 700 bp from the RFP (Ring finger protein) gene) containing different cytosine modifications. The mC and hmC control DNA was generated by PCR with the corresponding nucleotide. The caC control fragment was obtained by in vitro methylation using M.SssI methyltransferase followed by oxidation with purified Tet2. The IP'd DNA was subsequently analyzed by qPCR using primers specific for the control DNA fragments and for GAPDH, used as a negative control. This figure shows the enrichment calculated as the ratio of the recovery of the control DNA versus the recovery of the GAPDH negative control.

Immunofluorescence Microscopy

Immunofluorescence results of Rabbit anti-5-Carboxylcytosine antibody. Tissue: 293T cells (mock), transfected with catalytically inactive FLAG-tagged C-terminal domain (Tet1 mCD), or transfected with mouse FLAG-tagged wild-type (Tet1 CD). Antigen retrieval: not required. Primary antibody: anti-5-Carboxylcytosine antibody at 1:500 for 1 hr at RT. Secondary antibody: rabbit anti-FLAG antibody at 1:10,000 for 45 min at RT. Staining: 5-caC antibody as (red) fluorescent signal, DAPI (blue), FLAG (in green).

Dot Blot

Dot Blot results of Rabbit anti-5-Carboxylcytosine antibody. Antigen: C, 5mC, 5hmC, 5fC, 5caC forms. Load: 125ng and 25ng. Plate Coated: Streptavidin. Primary antibody: 5-Carboxylcytosine antibody at 1:1,000.

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.