diagenode
A Hologic Company

TECHNICAL DATASHEET

PRODUCT NAME 5-caC polyclonal antibody		
Full name : 5-Carboxylcytosine polyclonal antibody		
Cat. No. C15410204-020 (pAb-caC-120) C15410204-100 (pAb-caC-100)	**Type:** Polyclonal	**Size:** 20 µg / 20 µl 100 µg / 100 µl
Lot #: 001	**Source:** Rabbit	**Concentration:** 1 µg/µl

Product description: Polyclonal antibody raised in rabbit against 5-Carboxylcytosine (5ca-CMP monophosphate) conjugated to BSA.

Specificity: Human, mouse, other (wide range): positive

Applications	Suggested dilution	References
Dot Blot	1:500 - 1:1,000	Fig 1
Immunofluorescence	1:500	Fig 2
IP*	4 µg/IP (4 µg genomic DNA per IP)	Fig 3

*Please note that of the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 µg per IP.

Purity: Protein A purified polyclonal antibody in PBS (pH 7.4) containing 0.05% sodium azide and 30% glycerol.

Storage: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.

Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Last data sheet update: March 27, 2013

Target description

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. We previously released highly specific antibodies directed against 5-mC and 5-hmC. Now, we also present a unique rabbit polyclonal antibody against 5-Carboxycytosine.

References citing this antibody:

(1) The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Kriaucionis S, Heintz N., Science. 2009 May 15;324(5929):929-30. Epub 2009 Apr 16.

(2) Tissue distribution of 5-hydroxymethylcytosine and search for active demethylation intermediates. Globisch D, Münzel M, Müller M, Michalakis S, Wagner M, Koch S, Brückl T, Biel M, Carell T., PLoS One. 2010 Dec 23;5(12):e15367.

(3) Thymine DNA glycosylase can rapidly excise 5-formylcytosine and 5-carboxylcytosine: potential implications for active demethylation of CpG sites. Maiti A, Drohat AC. ; J Biol Chem. 2011 Oct 14;286(41):35334-8. Epub 2011 Aug 23.

(4) The role of Tet3 DNA dioxygenase in epigenetic reprogramming by oocytes. Tian-Peng Gu, Fan Guo, Hui Yang, Jinsong Li and Guo-Liang Xu (2011) Nature, 477, 606-610.

(5) Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. Yu-Fei He, Bin-Zhong Li, Chuan He, Guo-Liang Xu (2011) Science 333, 1303–1307.

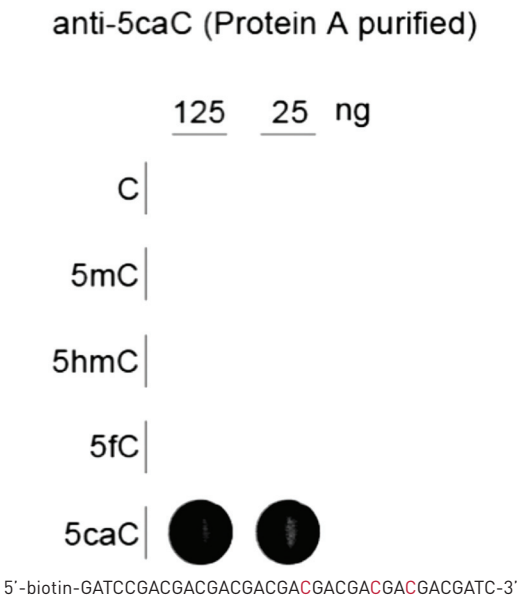

Fig. 1. Dot blot analysis using the Diagenode antibody directed against 5-caC

To demonstrate the specificity of the Diagenode antibody against 5-caC (cat. No. pAb-CaC-020/050), a Dot Blot analysis was performed using synthetic oligonucleotides containing different modified C-bases (indicated in red). 125 and 25 ng of the respective oligo's were bound to a Streptavidin-coated multi-well plate. The antibody was used at a dilution of 1:1,000. The binding of antibody to the DNA was measured by ECL chemiluminescence. Figure 1 shows a high specificity of the antibody for the carboxylated cytosine.

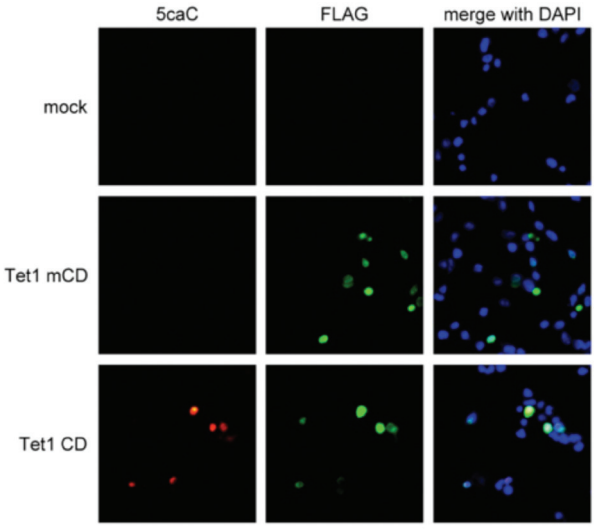

Fig. 2. Immunofluorescence assay using the Diagenode antibody directed against 5-caC

293T cells were transfected with either the mouse FLAG-tagged wild-type Tet1 (Tet1 CD) or the catalytically inactive FLAG-tagged C-terminal domain of Tet1 (Tet1 mCD) and stained with the Diagenode antibody against 5-caC (cat. No. pAb-CaC-020/050), diluted 1:500, and with an anti-FLAG antibody, followed by DAPI counterstaining.

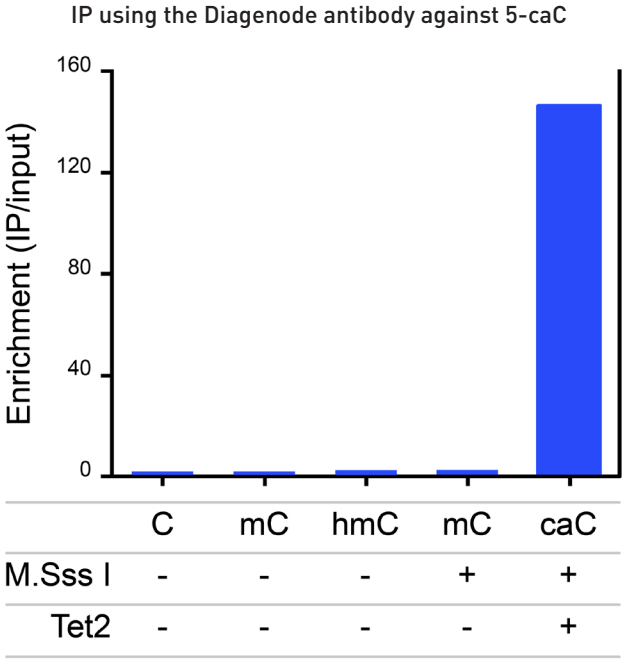

Fig. 3. Immunoprecipitation using the Diagenode antibody directed against 5-caC

Immunoprecipitation was performed with the Diagenode antibody against 5-caC (cat. No. pAb-CaC-020/050) 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 anaysed by qPCR using primers specific for the control DNA fragments and for GAPDH, used as a negative control. Figure 3 shows the enrichment calculated as the ratio of the recovery of the control DNA versus the recovery of the GAPDH negative control.