abcam

ab240289

Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade - BSA and Azide free

Recombinant | RabMAb | Advanced Validation | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR18894
Purification technique	Affinity purification Protein A
Concentration	0.993 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ChIC/CUT&RUN-seq

Tested

Species	Human
Dilution info	-

Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

ChIP-seq

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Human, Rat, Mouse
Dilution info	-
Notes	-

Target data

See full target information EHMT2

Function	Histone methyltransferase that specifically mono- and dimethylates 'Lys-9' of histone H3 (H3K9me1 and H3K9me2, respectively) in euchromatin. H3K9me represents a specific tag for epigenetic transcriptional repression by recruiting HP1 proteins to methylated histones (PubMed:11316813, PubMed:20084102). Also mediates monomethylation of 'Lys-56' of histone H3 (H3K56me1) in G1 phase, leading to promote interaction between histone H3 and PCNA and regulating DNA replication (PubMed:22387026). Also weakly methylates 'Lys-27' of histone H3 (H3K27me) (PubMed:11316813). Also required for DNA methylation, the histone methyltransferase activity is not required for DNA methylation, suggesting that these 2 activities function independently. Probably targeted to histone H3 by different DNA-binding proteins like E2F6, MGA, MAX and/or DP1. Also able to mono- and dimethylate histone H1-4 at 'Lys-26' (H1.4K26me1 and H1.4K26me2, respectively) (PubMed:19144645). In addition to the histone methyltransferase activity, also methylates non-histone proteins: mediates dimethylation of 'Lys-373' of p53/TP53 (PubMed:20118233). Also methylates CDYL, WIZ, ACIN1, DNMT1, HDAC1, ERCC6, KLF12 and itself (PubMed:18438403).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab240289 is the carrier-free version of ab185050.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	EHMT2 also known as G9a is a methyltransferase enzyme with a molecular mass of approximately 165 kDa. It modifies histones by adding methyl groups to lysine 9 on histone H3 which results in chromatin structure changes and influences gene expression. EHMT2/G9a expresses in various tissues including brain heart and liver showing its widespread role in different physiological processes. As a well-studied protein researchers often analyze EHMT2 activity using assays like ELISA and western blot.
Biological function summary	EHMT2/G9a regulates gene expression by altering chromatin accessibility. It is part of a chromatin remodeling complex interacting with proteins such as ANMTA02. Through its methyltransferase activity it maintains an epigenetic silence state important for embryonic development differentiation and cellular identity. EHMT2/G9a plays a significant role in the repression of transcriptional activity of tumor suppressor genes and developmental genes.
Pathways	G9a functions in epigenetic signaling pathways and influences processes like the cell cycle and DNA damage repair. It interacts with proteins in the Polycomb repressive complex such as EZH2 to regulate gene silencing. Additionally in the DNA damage response pathway G9a can associate with histone deacetylases to stabilize chromatin and prevent unwarranted gene expression.
Associated diseases and disorders	Aberrant regulation of EHMT2/G9a associates with cancer and neurodevelopmental disorders. In various cancers such as leukemia G9a suppresses tumor suppressor genes aiding tumor progression. Connections between G9a and proteins like DNMT1 highlight its role in maintaining a hypermethylation state in cancerous cells. In neurodevelopmental disorders

altered expression of G9a affects synaptic plasticity linking it with cognitive impairments and autism spectrum disorders.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

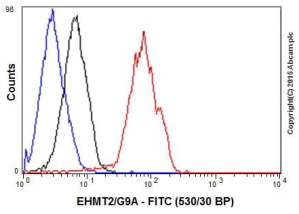
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

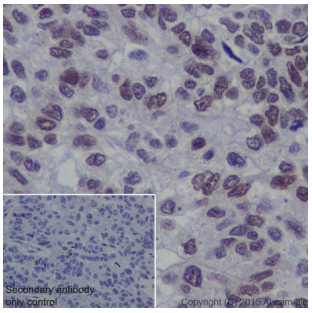
12 product images



Flow Cytometry (Intracellular) - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade - BSA and Azide free (ab240289)

EHMT2/G9A Flow Cytometry (Intracellular) staining using rabbit Anti-EHMT2/G9A antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling EHMT2/G9A with ab185050 at 1/150 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/150 dilution was used as the secondary antibody.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185050).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade - BSA and Azide free (ab240289)

EHMT2/G9A Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-EHMT2/G9A antibody

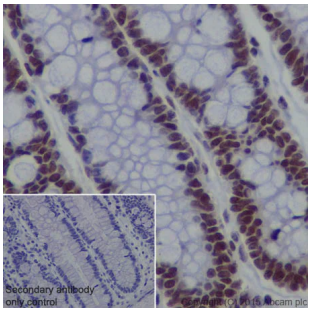
Immunohistochemical analysis of paraffin-embedded Human gastric adenocarcinoma tissue labeling EHMT2/G9A with ab185050 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.
Nucleus staining on tumor cells of the gastric adenocarcinoma is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185050).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade - BSA and Azide free (ab240289)

EHMT2/G9A Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-EHMT2/G9A antibody

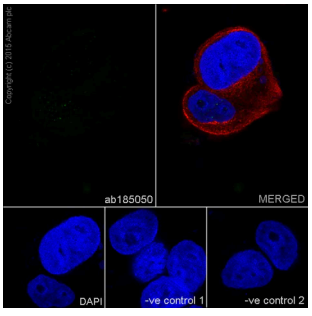
Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling EHMT2/G9A with ab185050 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.
Nucleus staining on epithelial cells of the normal Human colon is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185050).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade - BSA and Azide free (ab240289)

EHMT2/G9A Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-EHMT2/G9A antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized MCF7 (Human breast adenocarcinoma cell line) cells labeling EHMT2/G9A with ab185050 at 1/100

dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).
Confocal image showing no staining on MCF7 cell line, as MCF7 cells have a very low level expression of EHMT2/G9A.

The nuclear counterstain is DAPI (blue).

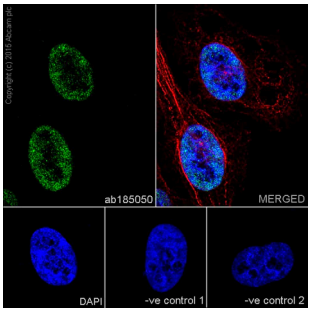
Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab185050 at 1/70 dilution followed by ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution.

-ve control 2: ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185050).



Immunocytochemistry/ Immunofluorescence - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade - BSA and Azide free (ab240289)

EHMT2/G9A Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-EHMT2/G9A antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling EHMT2/G9A with ab185050 at 1/1000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).
Confocal image showing nuclear staining on HeLa cell line.

The nuclear counterstain is DAPI (blue).

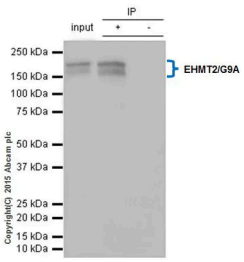
Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab185050 at 1/1000 dilution followed by ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution.

-ve control 2: ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185050).



Immunoprecipitation - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade - BSA and Azide free (ab240289)

EHMT2/G9A was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate with ab185050 at 1/50 dilution.
Western blot was performed from the immunoprecipitate using ab185050 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1/10000 dilution.

Lane 1: HeLa whole cell lysate 10ug (Input).

Lane 2: ab185050 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab185050 in HeLa whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

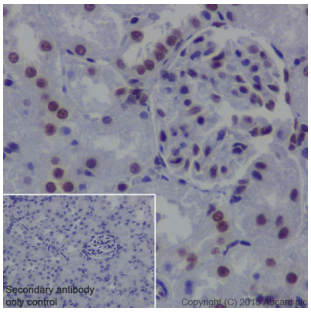
Exposure time: 30 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185050).

All lanes:
Immunoprecipitation - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade (ab185050)

Predicted band size: 132 kDa

Observed band size: 160 kDa, 170 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade - BSA and Azide free (ab240289)

EHMT2/G9A Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-EHMT2/G9A antibody

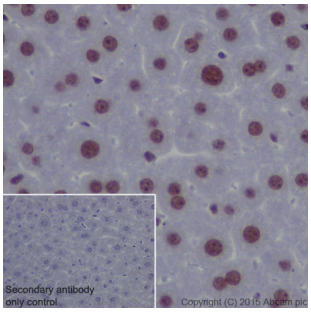
Immunohistochemical analysis of paraffin-embedded Rat kidney tissue labeling EHMT2/G9A with ab185050 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.
Nucleus staining on rat kidney is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185050).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade - BSA and Azide free (ab240289)

EHMT2/G9A Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-EHMT2/G9A antibody

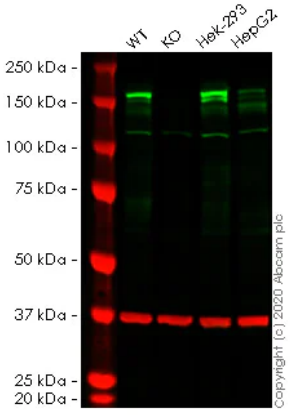
Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling EHMT2/G9A with ab185050 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.
Nucleus staining on hepatocytes of the mouse liver is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185050).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade - BSA and Azide free (ab240289)

EHMT2/G9A Western blot staining using rabbit Anti-EHMT2/G9A antibody

This data was developed using the same antibody clone in a different buffer formulation (ab185050).
Lanes 1-4: Merged signal (red and green). Green - ab185050 observed at 160 kDa. Red - loading control ab8245 observed at 37 kDa.

ab185050 Anti-EHMT2/G9A antibody [EPR18894] was shown to specifically react with EHMT2/G9A in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265149 (knockout cell lysate ab257080) was used. Wild-type and EHMT2/G9A knockout samples were subjected to SDS-PAGE. ab185050 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade (ab185050) at 1/1000 dilution

Lane 1:
Wild-type HeLa cell lysate at 20 µg

Lane 2:
Western blot - Human EHMT2 (G9A) knockout HeLa cell lysate (ab257080) at 20 µg

Lane 3:
HEK-293 cell lysate at 20 µg

Lane 4:
HepG2 cell lysate at 20 µg

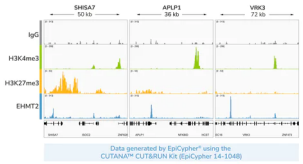
Secondary

All lanes:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 132 kDa

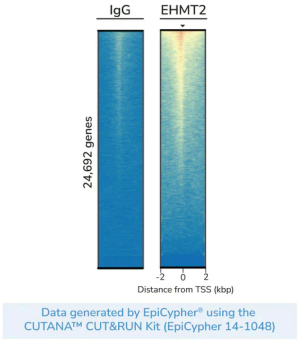
Observed band size: 160 kDa



ChIC/CUT&RUN sequencing - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade - BSA and Azide free (ab240289)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185050)

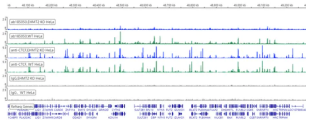
CUT&RUN profiling with EHMT2 antibody reveals the expected genomic enrichment pattern in cells. Representative genome browser tracks show CUT&RUN data generated using the CUTANA™ CUT&RUN Kit (EpiCypher 14-1048) with EHMT2 antibody (Abcam ab185050, 0.5 µg). 500,000 HeLa cells were used per reaction. IgG, H3K4me3, and H3K27me3 antibodies were included as controls to assess non-specific background, active promoters, and repressed chromatin, respectively. Libraries were prepared using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001). Sequencing was performed with paired-end 50 bp reads, and data were processed on CUTANA™ Cloud (cloud.epicypher.com) by alignment to the hg38 genome. Images were generated using Integrative Genomics Viewer (IGV, Broad Institute).



ChIC/CUT&RUN sequencing - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade - BSA and Azide free (ab240289)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185050)

CUT&RUN profiling with EHMT2 antibody demonstrates robust genome-wide enrichment in cells. Heatmaps of genome-wide signal flanking annotated transcription start sites (TSSs, +/- 2 kbp) display CUT&RUN data generated using the CUTANA™ CUT&RUN Kit (EpiCypher 14-1048) with EHMT2 antibody (Abcam ab185050, 0.5 µg). 500,000 HeLa cells were used per reaction. IgG antibody was included as a negative control to assess non-specific background. Libraries were prepared using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001). Sequencing was performed with paired-end 50 bp reads, and data were processed on CUTANA™ Cloud (cloud.epicypher.com) by alignment to the hg38 genome. Heatmaps were generated using ChAsE (Younesy et al., *Bioinformatics* 2016; PMID 27378294). Row-linked data are ranked by intensity relative to EHMT2, with red indicating high localized enrichment and blue denoting background.



ChIC/CUT&RUN sequencing - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade - BSA and Azide free (ab240289)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL. 2.5X10^5 of Human wild-type HeLa cell line (ab255928) or EHMT2 (G9A) knockout HeLa cell line (ab265149) were used along with 5µg of ab185050 [EPR18894]. Assay Quality Control was conducted using 5µg Anti-CTCF (ab188408) on the same cell lines. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control ab172730 is also shown. Additional screenshots of mapped reads can be found in the Protocol booklet in the Product Protocol section. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-

Cleavage) methods. This data was developed using the same antibody clone in a different buffer formulation (ab185050).

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.