abcam

Product datasheet

Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade ab185050

敲除验证 重组 RabMAb

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概述

产品名称	Anti-EHMT2/G9A抗体[EPR18894] - ChIP Grade
描述	兔单克隆抗体[EPR18894] to EHMT2/G9A - ChIP Grade
宿主	Rabbit
经测试应用	**适用于:** ChIP-sequencing, WB, IHC-P, ICC/IF, IP, Flow Cyt (Intra), ChIC/CUT&RUN-seq
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: HeLa, HEK-293, Jurkat, HepG2, NCCIT, F9, Neuro-2a, LLC1, C6, RAW 264.7, PC-12 and NIH/3T3 whole cell lysates. Human fetal heart and fetal kidney lysates. IHC-P: Human colon, Human gastric adenocarcinoma, mouse liver and rat kidney tissues. ICC/IF: HeLa. Flow Cyt (intra): HeLa cells. IP: HeLa whole cell lysate. ChIP-seq: HeLa cells.
常规说明	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 production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR18894

同种型 IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab185050于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ChIP-sequencing		Use 8µg for 10^7 cells.
WB	★★★★★ (1)	1/1000. Detects a band of approximately 170, 160 kDa (predicted molecular weight: 132 kDa).
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF	★★★★★ (1)	1/1000.
IP	★★★★☆ (1)	1/50.
Flow Cyt (Intra)		1/150.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5µg

靶标

功能 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. 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. Also weakly methylates 'Lys-27' of histone H3 (H3K27me). 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. May also methylate histone H1. In addition to the histone methyltransferase activity, also methylates non-histone proteins: mediates dimethylation of 'Lys-373' of p53/TP53. Also methylates CDYL, WIZ, ACIN1, DNMT1, HDAC1, ERCC6, KLF12 and itself.

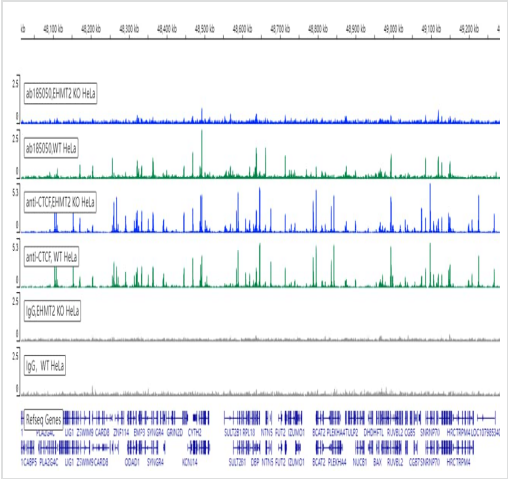
组织特异性 Expressed in all tissues examined, with high levels in fetal liver, thymus, lymph node, spleen and peripheral blood leukocytes and lower level in bone marrow.

序列相似性 Belongs to the class V-like SAM-binding methyltransferase superfamily. Histone-lysine methyltransferase family. Suvar3-9 subfamily.
Contains 7 ANK repeats.
Contains 1 post-SET domain.
Contains 1 pre-SET domain.
Contains 1 SET domain.

结构域 The SET domain mediates interaction with WIZ.

	The ANK repeats bind H3K9me1 and H3K9me2.
翻译后修饰	Methylated at Lys-185; automethylated.
细胞定位	Nucleus. Chromosome. Associates with euchromatic regions. Does not associate with heterochromatin.

图片

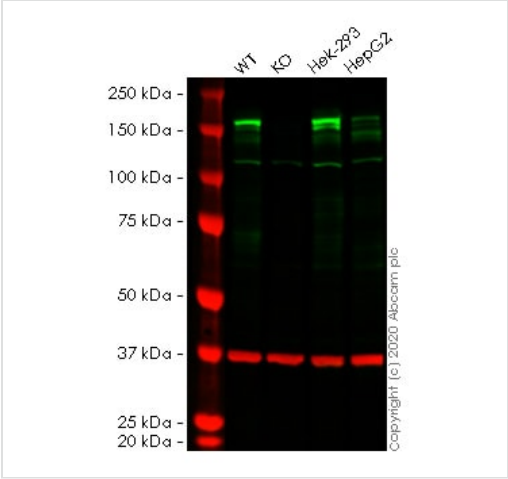


ChIC/CUT&RUN sequencing - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade (ab185050)

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 downloaded **here**.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



Western blot - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade (ab185050)

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

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : EHMT2/G9A knockout HeLa cell lysate
Lane 3 : HEK-293 cell lysate
Lane 4 : HepG2 cell lysate

Lysates/proteins at 20 µg per lane.

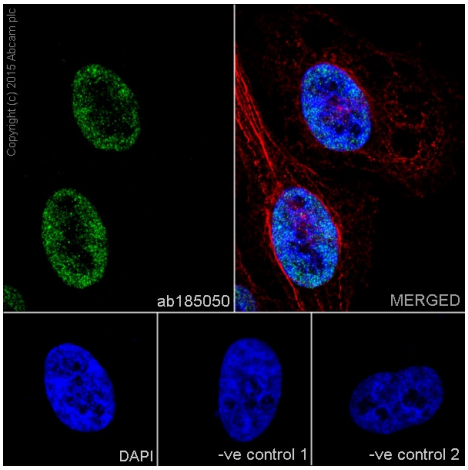
Performed under reducing conditions.

Predicted band size: 132 kDa

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.



Immunocytochemistry/ Immunofluorescence - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade (ab185050)

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.

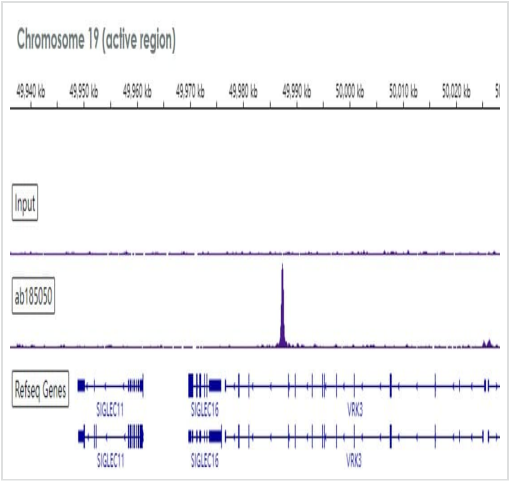
The nuclear counterstain is DAPI (blue).

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.

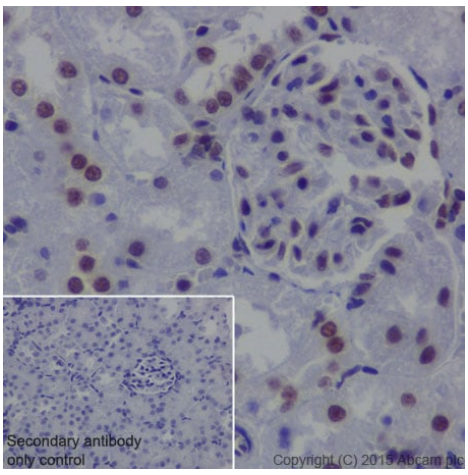
-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.



ChIP-sequencing - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade (ab185050)

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 HeLa cells and 8 µg of ab185050 [EPR18894]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

Additional screenshots of mapped reads can be downloaded **here**.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade (ab185050)

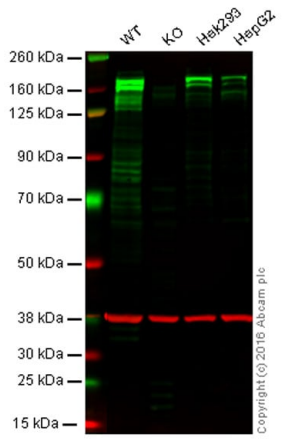
Immunohistochemical analysis of paraffin-embedded Rat kidney tissue labeling EHMT2/G9A with ab185050 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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.

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 (ab185050)

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

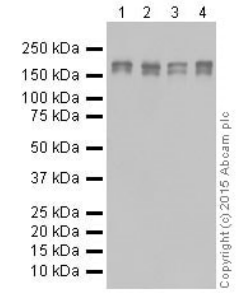
Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : EHMT2/G9A knockout HAP1 cell lysate
Lane 3 : HEK293 cell lysate
Lane 4 : HepG2 cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 132 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab185050 observed at 160 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab185050 was shown to recognize EHMT2/G9A when EHMT2/G9A knockout samples were used, along with additional cross-reactive bands. Wild-type and EHMT2/G9A knockout samples were subjected to SDS-PAGE. ab185050 and **ab8245** (loading control to GAPDH) were diluted 1/1000 and 1/10 000 respectively and incubated overnight at 4°C. 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 1/10 000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade (ab185050)

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

Lane 1 : HEK-293 (Human epithelial cells from embryonic kidney) whole cell lysate
Lane 2 : Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate
Lane 3 : HepG2 (Human liver hepatocellular carcinoma) whole cell lysate
Lane 4 : NCCIT (Human pluripotent embryonic carcinoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

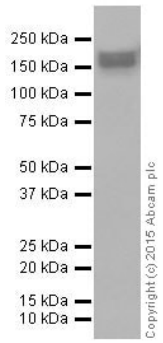
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 132 kDa
Observed band size: 160,170 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID 16702210).



Western blot - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade (ab185050)

Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade (ab185050) at 1/1000 dilution + Human fetal heart lysate at 10 µg

Secondary

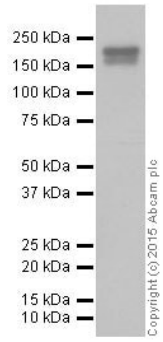
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 132 kDa
Observed band size: 160,170 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID 16702210).



Western blot - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade (ab185050)

Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade (ab185050) at 1/1000 dilution + Human fetal kidney lysate at 10 µg

Secondary

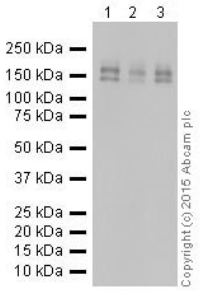
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 132 kDa
Observed band size: 160,170 kDa

Exposure time: 8 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID 16702210).



Western blot - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade (ab185050)

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

Lane 1 : F9 (Mouse embyro testicular cancer cell line) whole cell lysate
Lane 2 : Neuro-2a (Mouse neuroblastoma cells) whole cell lysate
Lane 3 : LLC1 (Mouse lung carcinoma cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

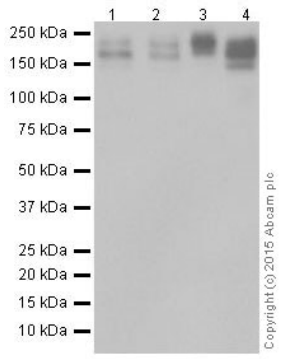
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 132 kDa
Observed band size: 160,170 kDa

Exposure time: 5 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID 16702210).



Western blot - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade (ab185050)

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

Lane 1 : C6 (Rat glial tumor cells) whole cell lysate
Lane 2 : RAW 264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) whole cell lysate
Lane 3 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate
Lane 4 : NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate

Lysates/proteins at 10 µg per lane.

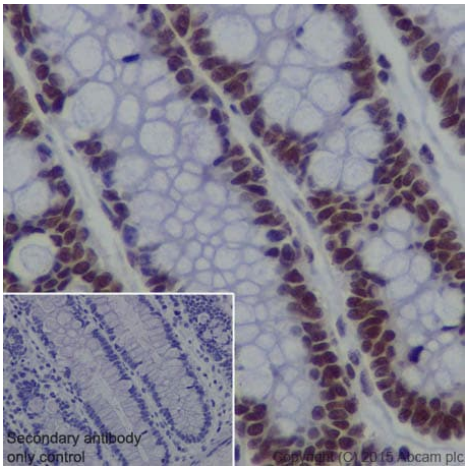
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 132 kDa
Observed band size: 160,170 kDa

Exposure time: 8 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID 16702210).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade (ab185050)

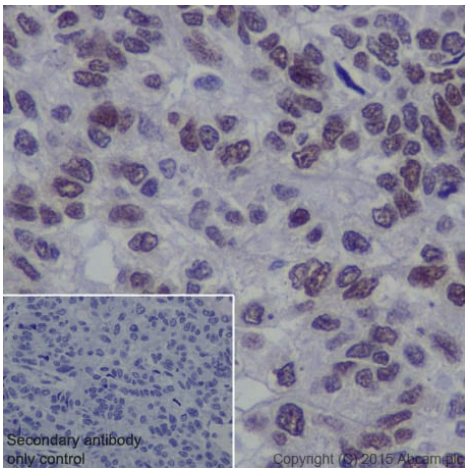
Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling EHMT2/G9A with ab185050 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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.

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 (ab185050)

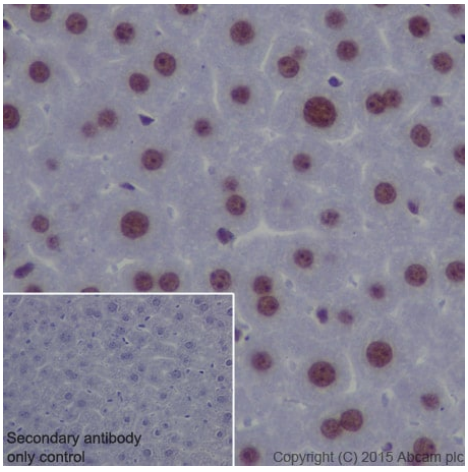
Immunohistochemical analysis of paraffin-embedded Human gastric adenocarcinoma tissue labeling EHMT2/G9A with ab185050 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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.

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 (ab185050)

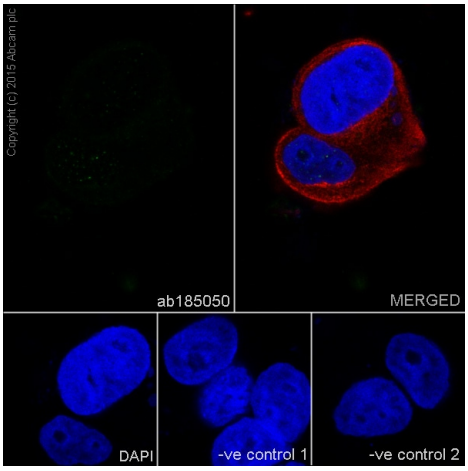
Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling EHMT2/G9A with ab185050 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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.

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 (ab185050)

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.

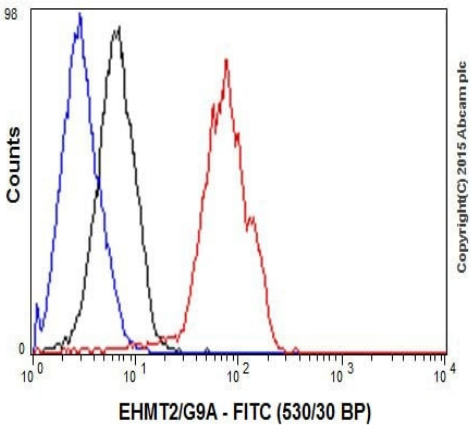
The nuclear counterstain is DAPI (blue).

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:-

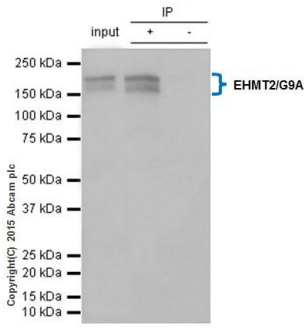
-ve control 1: ab185050 at 1/70 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution.

-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.



Flow Cytometry (Intracellular) - Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade (ab185050)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling EHMT2/G9Awith 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.



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

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.

Anti-EHMT2/G9A antibody [EPR18894] - ChIP Grade (ab185050)

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