abcam

Product datasheet

Anti-EHMT2/G9A + EHMT1/GLP antibody [EPR18667] ab194299

KO VALIDATED Recombinant RabMAb

10 Images

Overview

Product name	Anti-EHMT2/G9A + EHMT1/GLP antibody [EPR18667]
Description	Rabbit monoclonal [EPR18667] to EHMT2/G9A + EHMT1/GLP
Host species	Rabbit
Specificity	Not suitable for IP-Human and IP-Mouse.
Tested applications	**Suitable for:** IHC-P, WB, ICC/IF, Flow Cyt (Intra) **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human fetal kidney lysate; HEK-293, HeLa, K562, MCF7 and PC-3 whole cell lysates; EHMT2 recombinant protein, Wild-type HAP1 cell lysate, HEK-293T; NIH/3T3, PC-12 and Raw 264.7 cell lysates. Flow Cyt (Intra): HeLa cells. IHC-P: Human tonsil tissue; ICC/IF: HeLa and NIH/3T3 cells.
General notes	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**.

Properties

Form	Liquid
Storage instructions	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.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide

	Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18667
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab194299 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

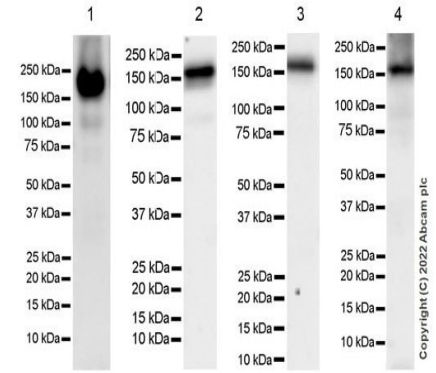
Application	Abreviews	Notes
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 170 kDa (predicted molecular weight: 141, 132 kDa).
ICC/IF		1/1000.
Flow Cyt (Intra)		Use at an assay dependent concentration.

Application notes Is unsuitable for IP.

Target

Cellular localization EHMT2/G9A: Nucleus. Chromosome. Associates with euchromatic regions. Does not associate with heterochromatin. EHMT1/GLP: Nucleus. Chromosome. Associates with euchromatic regions.

Images



Western blot - Anti-EHMT2/G9A + EHMT1/GLP antibody [EPR18667] (ab194299)

All lanes : Anti-EHMT2/G9A + EHMT1/GLP antibody [EPR18667] (ab194299) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : NIH/3T3 (mouse embryonic fibroblast) whole cell lysate
Lane 3 : PC-12 (rat adrenal gland pheochromocytoma cell) whole cell lysate
Lane 4 : Raw 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) 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

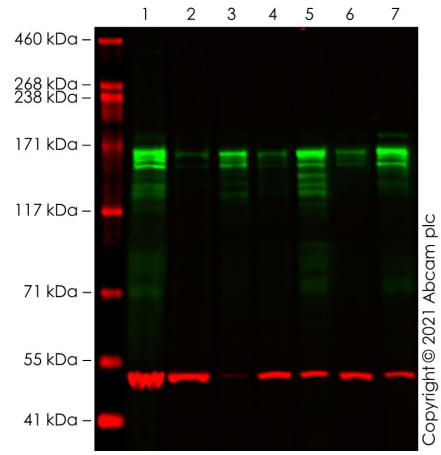
Predicted band size: 141, 132 kDa
Observed band size: 170 kDa

Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM/TBST

Exposure Time: Lane 1: 15 seconds, Lane 2-4: 37 seconds.

Lysates were freshly made and used for Western blotting immediately to minimize protein degradation.



Western blot - Anti-EHMT2/G9A + EHMT1/GLP antibody [EPR18667] (ab194299)

All lanes : Anti-EHMT2/G9A + EHMT1/GLP antibody [EPR18667] (ab194299) at 1/1000 dilution

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : EHMT1 knockout HAP1 cell lysate
Lane 3 : Wild-type HAP1 Nuclear cell lysate
Lane 4 : HeLa cell lysate
Lane 5 : HeLa Nuclear cell lysate
Lane 6 : Wild-type HEK-293T cell lysate
Lane 7 : Wild-type HEK-293T nuclear cell lysate

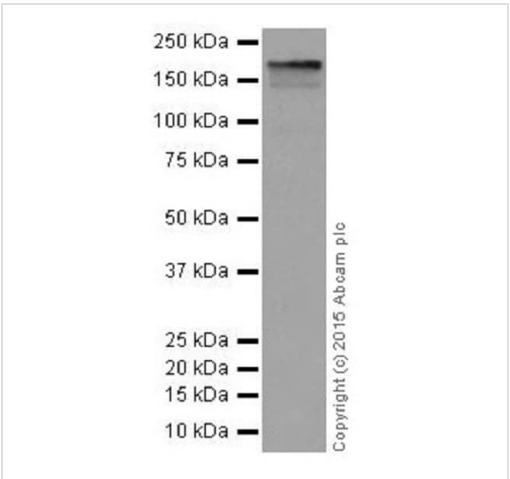
Lysates/proteins at 40 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW)

preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preabsorbed (**ab216776**) at 1/20000 dilution

Predicted band size: 141, 132 kDa
Observed band size: 150-170 kDa

False colour image of Western blot: Anti-EHMT2/G9A + EHMT1/GLP antibody [EPR18667] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab194299 was shown to bind specifically to EHMT1/GLP and EHMT2/G9A. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-EHMT2/G9A + EHMT1/GLP antibody [EPR18667] (ab194299)

Anti-EHMT2/G9A + EHMT1/GLP antibody [EPR18667] (ab194299) at 1/1000 dilution + Human fetal kidney lysate at 10 µg

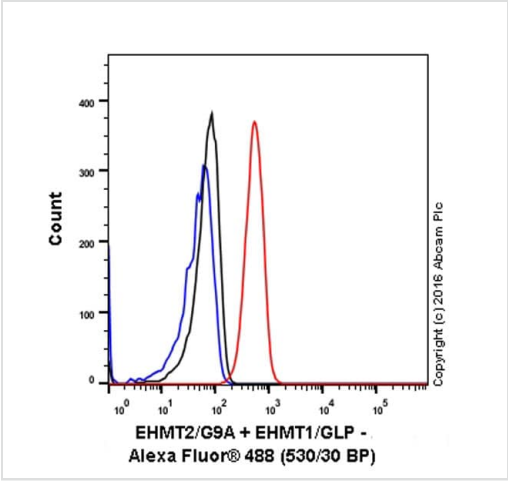
Secondary
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/10000 dilution

Developed using the ECL technique.

Predicted band size: 141, 132 kDa
Observed band size: 170 kDa

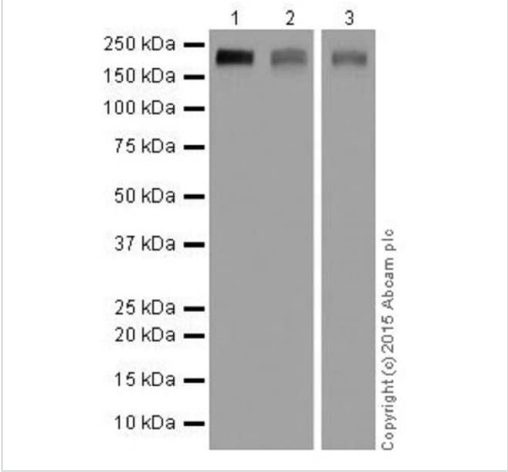
Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-EHMT2/G9A + EHMT1/GLP antibody [EPR18667] (ab194299)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labelling EHMT2/G9A + EHMT1/GLP (red) with purifiedab194299at a dilution of 1/150. Goat anti rabbit IgG (Alexa Fluor® 488) was used as the secondary antibody at 1/2000. Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Isotype control antibody was Rabbit monoclonal IgG (black). The blue line shows cells without incubation with primary antibody and secondary antibody.



Western blot - Anti-EHMT2/G9A + EHMT1/GLP antibody [EPR18667] (ab194299)

All lanes : Anti-EHMT2/G9A + EHMT1/GLP antibody [EPR18667] (ab194299) at 1/5000 dilution

Lane 1 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 3 : K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate

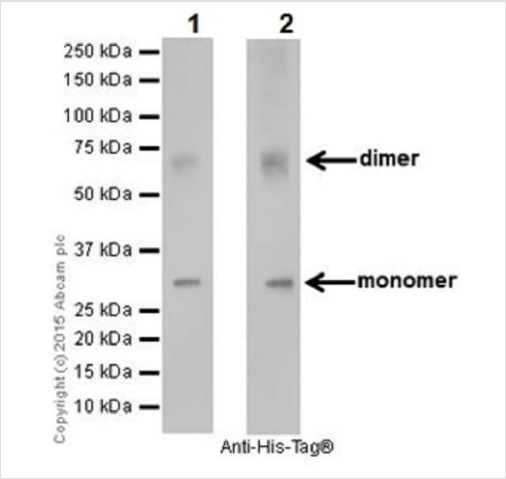
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 141, 132 kDa
Observed band size: 170 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lanes 1 & 2: 20 seconds; Lane 3: 30 seconds.



Western blot - Anti-EHMT2/G9A + EHMT1/GLP antibody [EPR18667] (ab194299)

Lane 1 : Anti-EHMT2/G9A + EHMT1/GLP antibody [EPR18667] (ab194299) at 1/5000 dilution
Lane 2 : Anti His-Tag®.

All lanes : EHMT2 recombinant protein

Lysates/proteins at 0.01 µg per lane.

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

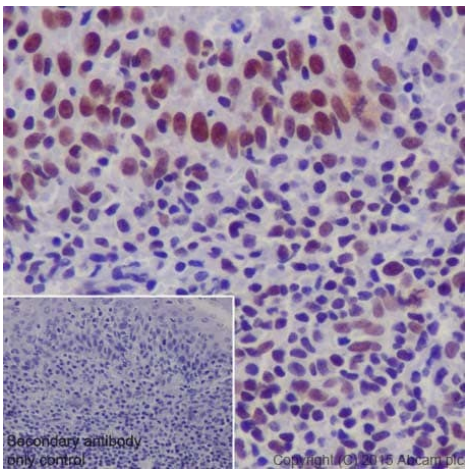
Developed using the ECL technique.

Predicted band size: 141, 132 kDa
Observed band size: 30,60 kDa

Exposure time: 5 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

EHMT2 recombinant protein fragment with His-Tag®.

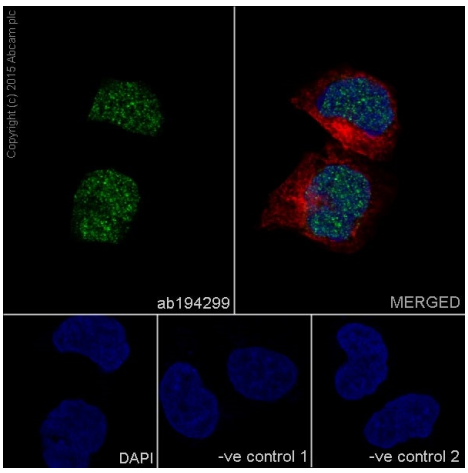


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EHMT2/G9A + EHMT1/GLP antibody [EPR18667] (ab194299)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling EHMT2/G9A + EHMT1/GLP with ab194299 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Nuclear staining on human tonsil 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.

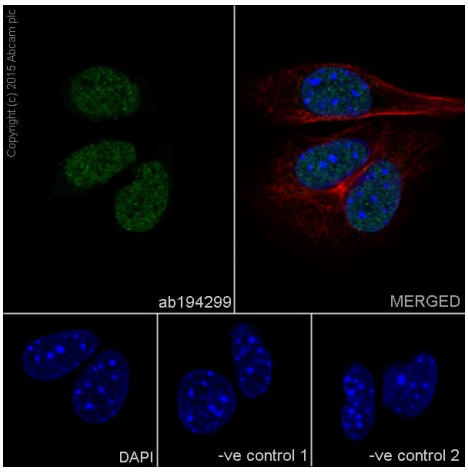


Immunocytochemistry/ Immunofluorescence - Anti-EHMT2/G9A + EHMT1/GLP antibody [EPR18667] (ab194299)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling EHMT2/G9A + EHMT1/GLP with ab194299 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 counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution (red).

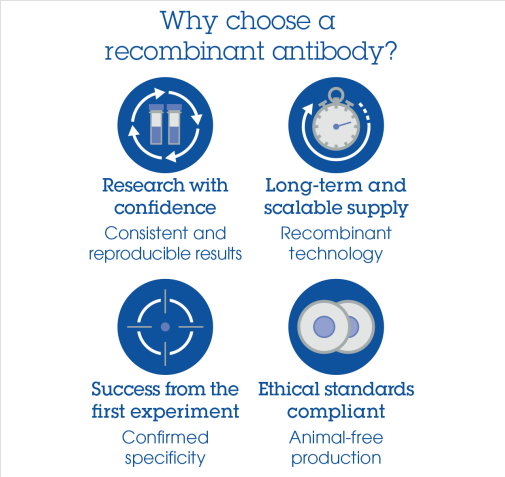
The negative controls are as follows:
-ve control 1: ab194299 at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) at 1/1000 dilution.
-ve control 2: Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-EHMT2/G9A + EHMT1/GLP antibody [EPR18667] (ab194299)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% tritonX-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling EHMT1/GLP + EHMT2/G9A with ab194299 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 NIH/3T3. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution (red).
The negative controls are as follows:
-ve control 1: ab194299 at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary at 1/1000 dilution.
-ve control 2: Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary at 1/1000 dilution.



Anti-EHMT2/G9A + EHMT1/GLP antibody [EPR18667] (ab194299)

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