abcam

Product datasheet

Anti-Dnmt3a antibody [EPR18455] ab188470

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Dnmt3a antibody [EPR18455]
Description	Rabbit monoclonal [EPR18455] to Dnmt3a
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, ICC/IF, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, HEK-293 and C6 cell lysates; Rat brain and heart lysates. ICC/IF: HeLa cells. IHC-P: Human placenta, mouse testis, rat spleen tissue
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: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18455
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab188470 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
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB	★★★★☆ **(2)**	1/2000. Detects a band of approximately 130 kDa (predicted molecular weight: 102 kDa).
ICC/IF		1/1000.
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target

Function Required for genome wide de novo methylation and is essential for the establishment of DNA methylation patterns during development. DNA methylation is coordinated with methylation of histones. It modifies DNA in a non-processive manner and also methylates non-CpG sites. May preferentially methylate DNA linker between 2 nucleosomal cores and is inhibited by histone H1. Plays a role in paternal and maternal imprinting. Required for methylation of most imprinted loci in germ cells. Acts as a transcriptional corepressor for ZNF238. Can actively repress transcription through the recruitment of HDAC activity.

Tissue specificity Highly expressed in fetal tissues, skeletal muscle, heart, peripheral blood mononuclear cells, kidney, and at lower levels in placenta, brain, liver, colon, spleen, small intestine and lung.

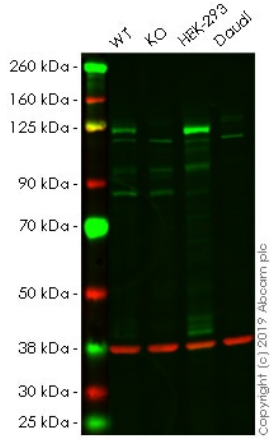
Sequence similarities Belongs to the C5-methyltransferase family.
Contains 1 ADD domain.
Contains 1 GATA-type zinc finger.
Contains 1 PHD-type zinc finger.
Contains 1 PWWP domain.

Domain The PWWP domain is essential for targeting to pericentric heterochromatin.

Post-translational modifications Sumoylated; sumoylation disrupts the ability to interact with histone deacetylases (HDAC1 and HDAC2) and repress transcription.

Cellular localization Nucleus. Cytoplasm. Accumulates in the major satellite repeats at pericentric heterochromatin.

Images



Western blot - Anti-Dnmt3a antibody [EPR18455] (ab188470)

All lanes : Anti-Dnmt3a antibody [EPR18455] (ab188470) at 1/2000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : Dnmt3a knockout HeLa cell lysate
Lane 3 : HEK-293 cell lysate
Lane 4 : Daudi cell lysate

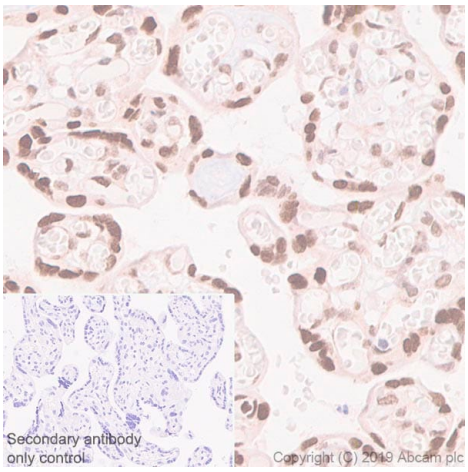
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 102 kDa

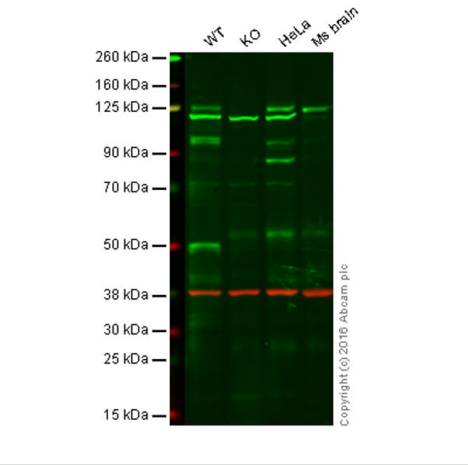
Lanes 1-4: Merged signal (red and green). Green - ab188470 observed at 125 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab188470 Anti-Dnmt3a antibody [EPR18455] was shown to specifically react with Dnmt3a in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab261793** (knockout cell lysate **ab257128**) was used. Wild-type and Dnmt3a knockout samples were subjected to SDS-PAGE. ab188470 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 2000 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Dnmt3a antibody [EPR18455] (ab188470)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human placenta tissue sections labeling Dnmt3a with purified ab188470 at 1/2000 (0.409 µg/ml). Antigen retrieval was heat mediated using **ab93684** (Tris/EDTA buffer, pH 9.0). Goat Anti-Rabbit IgG H&L (HRP) was used as the secondary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-Dnmt3a antibody [EPR18455] (ab188470)

All lanes : Anti-Dnmt3a antibody [EPR18455] (ab188470) at 1/5000 dilution

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : Dnmt3a knockout HAP1 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : Mouse brain tissue lysate

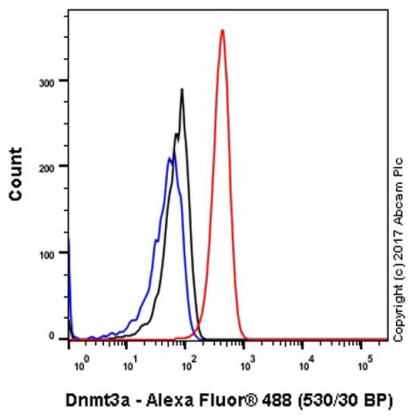
Lysates/proteins at 20 µg per lane.

Predicted band size: 102 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab188470 observed at 125 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

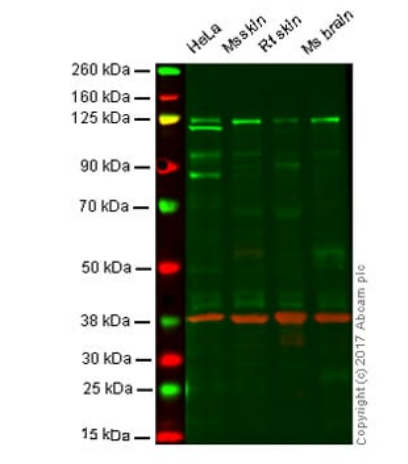
ab188470 was shown to recognize Dnmt3a when Dnmt3a knockout samples were used, along with additional cross-reactive bands. Wild-type and Dnmt3a knockout samples were subjected to SDS-PAGE. ab188470 and **ab8245** (loading control to GAPDH) were diluted to 1/5000 and 1/10000 respectively and incubated 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 1/10000 dilution for 1 hour at room temperature before imaging.



Flow Cytometry (Intracellular) - Anti-Dnmt3a antibody [EPR18455] (ab188470)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling Dnmt3a with purified ab188470 at 1/80 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluorr® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



Western blot - Anti-Dnmt3a antibody [EPR18455] (ab188470)

All lanes : Anti-Dnmt3a antibody [EPR18455] (ab188470) at 1/5000 dilution

Lane 1 : HeLa whole cell lysate
Lane 2 : Mouse skin tissue lysate
Lane 3 : Rat skin tissue lysate
Lane 4 : Mouse brain tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

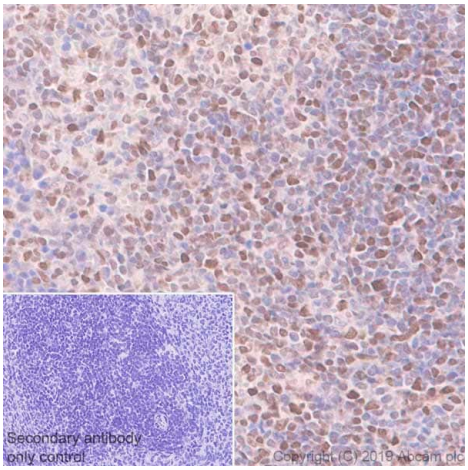
Performed under reducing conditions.

Predicted band size: 102 kDa
Observed band size: 125 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab188470 observed at 125 kDa. Red - loading control, **ab8245**, observed at

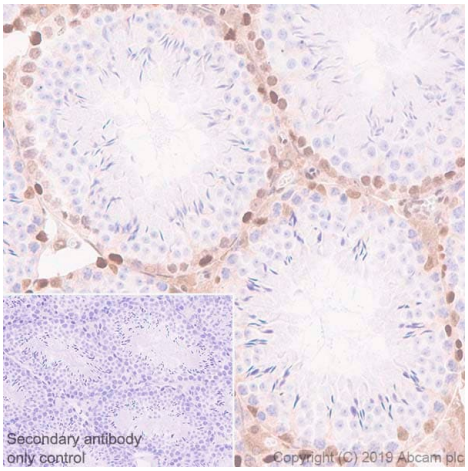
37 kDa.

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using Licor blocking buffer before being incubated with ab188470 and **ab8245** (loading control) overnight at 4°C. Antibody binding was detected using Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at a 1:10000 dilution for 1hr at room temperature and then imaged.



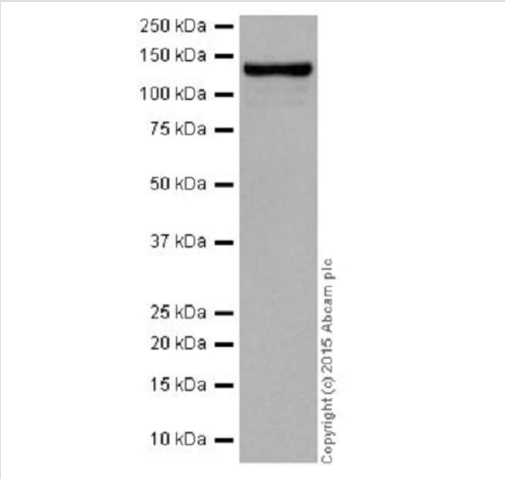
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Dnmt3a antibody [EPR18455] (ab188470)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat spleen tissue sections labeling Dnmt3 with purified ab188470 at 1/2000 (0.409 µg/ml). Antigen retrieval was heat mediated using **ab93684** (Tris/EDTA buffer, pH 9.0). Goat Anti-Rabbit IgG H&L (HRP) was used as the secondary antibody. Hematoxylin was used as a counterstain.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Dnmt3a antibody [EPR18455] (ab188470)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse testis tissue sections labeling Dnmt3a with purified ab188470 at 1/2000 (0.409 µg/ml). Antigen retrieval was heat mediated using **ab93684** (Tris/EDTA buffer, pH 9.0). Goat Anti-Rabbit IgG H&L (HRP) was used as the secondary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-Dnmt3a antibody [EPR18455] (ab188470)

Anti-Dnmt3a antibody [EPR18455] (ab188470) at 1/10000 dilution + HeLa (Human epithelial cells from cervix adenocarcinoma) cell lysate at 20 µg

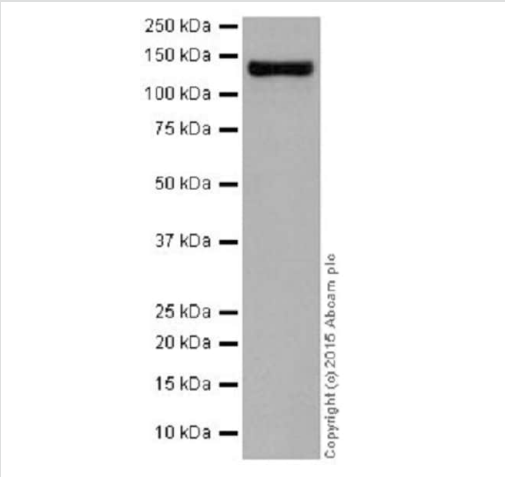
Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 102 kDa
Observed band size: 130 kDa

Exposure time: 5 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The observed MW is consistent with what has been described in the literature (J Biol Chem. 2002. 277, 38746-38754. PMID: 2138111).



Western blot - Anti-Dnmt3a antibody [EPR18455] (ab188470)

Anti-Dnmt3a antibody [EPR18455] (ab188470) at 1/10000 dilution + HEK-293 (Human epithelial cells from embryonic kidney) cell lysate at 20 µg

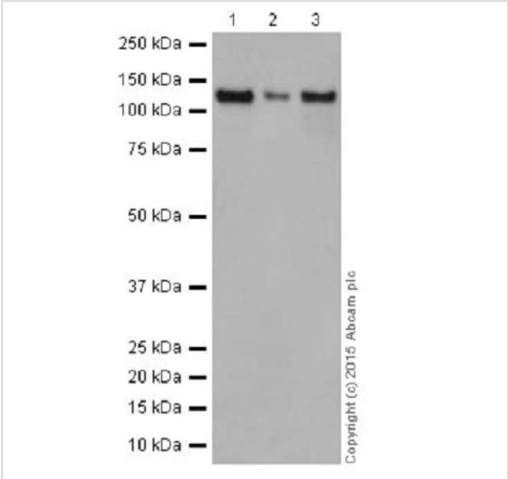
Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 102 kDa
Observed band size: 130 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The observed MW is consistent with what has been described in the literature (J Biol Chem. 2002. 277, 38746-38754. PMID: 2138111).



Western blot - Anti-Dnmt3a antibody [EPR18455] (ab188470)

All lanes : Anti-Dnmt3a antibody [EPR18455] (ab188470) at 1/2000 dilution

Lane 1 : Rat brain lysate
Lane 2 : Rat heart lysate
Lane 3 : C6 (Rat glial tumor cells) cell lysate

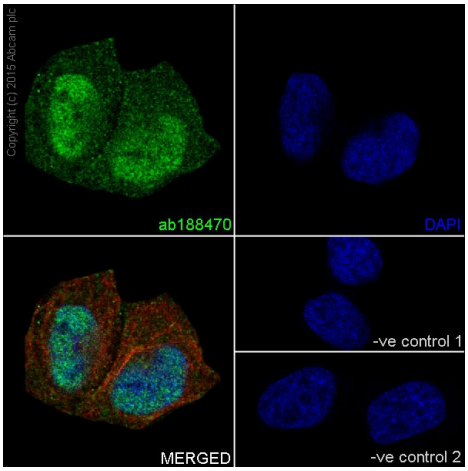
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 102 kDa
Observed band size: 130 kDa

Exposure time: 30 seconds

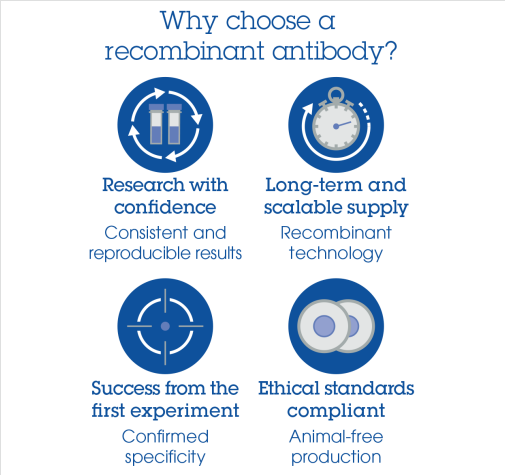
Blocking/Dilution buffer: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-Dnmt3a antibody [EPR18455] (ab188470)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Dnmt3a with ab188470 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 and weakly cytoplasmic staining on HeLa cell line. The nuclear counter stain 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: ab188470 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.



Anti-Dnmt3a antibody [EPR18455] (ab188470)

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