abcam

Product datasheet

Anti-Dnmt3a antibody [EPR18455-94] ab227823

KO VALIDATED Recombinant RabMAb

1 References 8 Images

Overview

Product name	Anti-Dnmt3a antibody [EPR18455-94]
Description	Rabbit monoclonal [EPR18455-94] to Dnmt3a
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P **Unsuitable for:** ChIP,Flow Cyt,ICC/IF or IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1, HEK-293T, HeLa and NIH/3T3 whole cell lysates; rat brain lysate. IHC-P: Human breast carcinoma and placenta tissue; mouse colon tissue; rat colon 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, 0.05% BSA, 40% Glycerol (glycerin, glycerine)
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18455-94

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab227823 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
WB		1/1000. Detects a band of approximately 125 kDa (predicted molecular weight: 102 kDa).
IHC-P		1/200. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Application notes Is unsuitable for ChIP,Flow Cyt,ICC/IF or IP.

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.

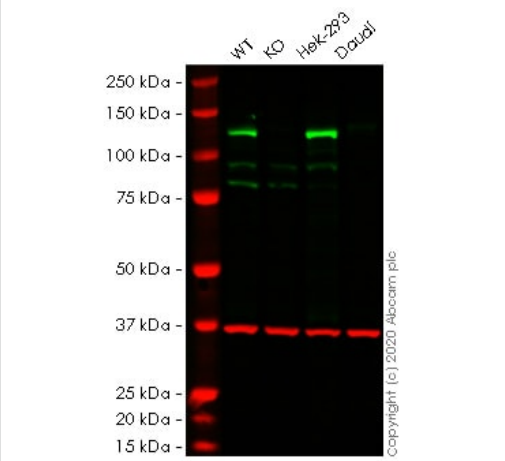
Sequence similarities Belongs to the C5-methyltransferase family.
Contains 1 ADD domain.
Contains 1 GATA-type zinc finger.
Contains 1 PHD-type zinc finger.
Contains 1 PWWP domain.

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 [EPR18455-94] (ab227823)

All lanes : Anti-Dnmt3a antibody [EPR18455-94] (ab227823) at 1/1000 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

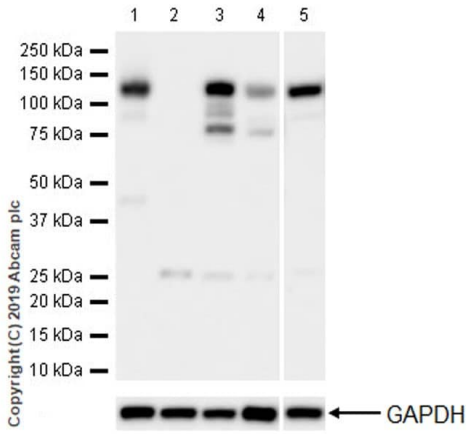
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 102 kDa

Lanes 1-4: Merged signal (red and green). Green - ab227823 observed at 125 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab227823 Anti-Dnmt3a antibody [EPR18455-94] 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. ab227823 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.



Western blot - Anti-Dnmt3a antibody [EPR18455-94] (ab227823)

All lanes : Anti-Dnmt3a antibody [EPR18455-94] (ab227823) at 1/1000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : Dnmt3a knockout HAP1 whole cell lysate
Lane 3 : HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate
Lane 4 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 5 : NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

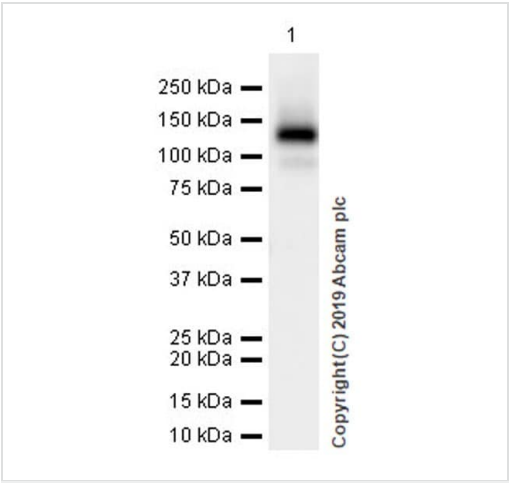
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 102 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

ab227823 was shown to specifically react with wild-type HAP1 cells as signal was lost in Dnmt3a knockout cells. Wild-type and Dnmt3a knockout samples were subjected to SDS-PAGE. ab227823 and **ab181602** (Rabbit anti-GAPDH loading control) were incubated 1 hour at room temperature at 1/1000 dilution and 1/200,000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) secondary antibody at 1/100,000 dilution for 1 hour at room temperature before imaging. The blot was developed on a BIO-RAD® ChemiDoc™ MP instrument using the ECL technique.

The observed bands ranging from 75~125kDa represent different Dnmt3a isoforms.



Western blot - Anti-Dnmt3a antibody [EPR18455-94] (ab227823)

Anti-Dnmt3a antibody [EPR18455-94] (ab227823) at 1/1000 dilution + Rat brain lysate at 20 µg

Secondary

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

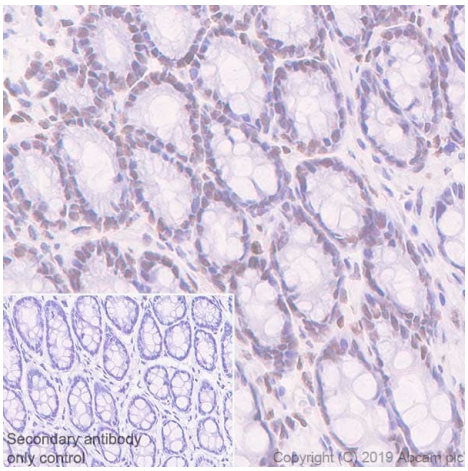
Predicted band size: 102 kDa

Observed band size: 125 kDa

Exposure time: 26 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

The observed bands ranging from 75~125kDa represent different Dnmt3a isoforms.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Dnmt3a antibody [EPR18455-94] (ab227823)

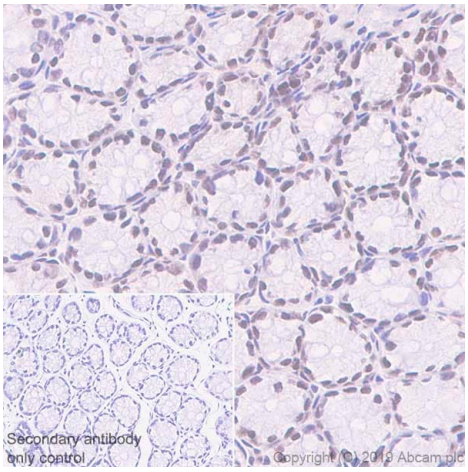
Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling Dnmt3a with ab227823 at 1/200 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on the rat colon (PMID: 24837369) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 minutes.

The section was incubated with ab227823 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Dnmt3a antibody [EPR18455-94] (ab227823)

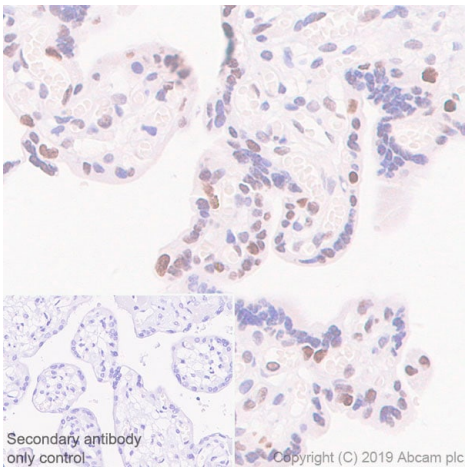
Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling Dnmt3a with ab227823 at 1/200 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on the mouse colon (PMID: 24837369) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 minutes.

The section was incubated with ab227823 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Dnmt3a antibody [EPR18455-94] (ab227823)

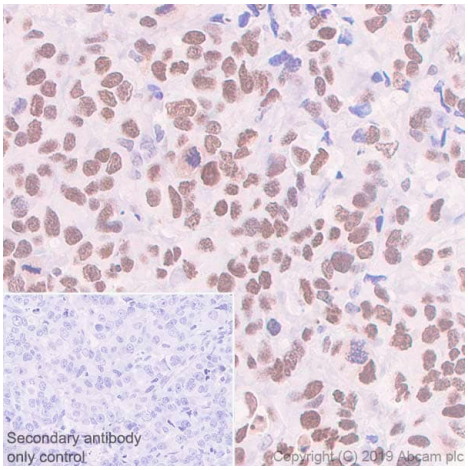
Immunohistochemical analysis of paraffin-embedded human placenta tissue labeling Dnmt3a with ab227823 at 1/200 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on the human placenta (PMID: 22413869) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 minutes.

The section was incubated with ab227823 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Dnmt3a antibody [EPR18455-94] (ab227823)

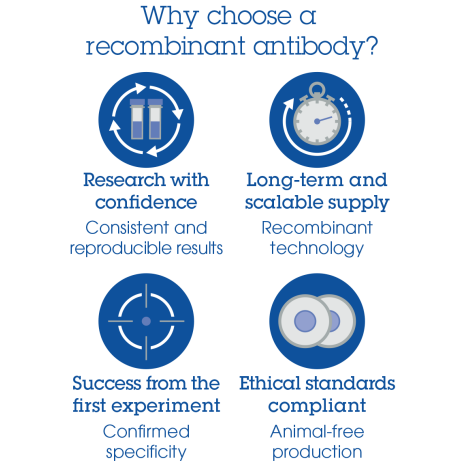
Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue labeling Dnmt3a with ab227823 at 1/200 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on the human breast carcinoma (PMID: 24464625) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 minutes.

The section was incubated with ab227823 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Anti-Dnmt3a antibody [EPR18455-94] (ab227823)

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