abcam

Product datasheet

Anti-Dnmtl antibody ab19905





8 Images

Overview

Product name Anti-Dnmt1 antibody

Description Rabbit polyclonal to Dnmt1

Host species Rabbit

Specificity Replenishment batches of our polyclonal antibody, ab19905 are tested in WB. Previous batches

> were additionally validated in ICC/IF, IHC-P and IP. These applications are still expected to work and are covered by our Abpromise guarantee. You may also be interested in our alternative

recombinant antibody, ab188453.

Tested applications Suitable for: WB, ICC/IF, IP, IHC-P

Species reactivity Reacts with: Human

Predicted to work with: Mouse

Immunogen Synthetic peptide conjugated to KLH derived from within residues 100 - 200 of Human Dnmt1.

Read Abcam's proprietary immunogen policy (Peptide available as ab21999.)

Positive control This antibody gave a positive signal in: (WB) HeLa nuclear; (IF) Hek293; HepG2; MCF7

General notes The immunogen used to generate this antibody has 71% identity with the corresponding region in

mouse Dnmt1. We have had variable feedback about the ability of this antibody to recognise mouse Dnmt1. Customers may prefer to use one of our other antibodies for detection of mouse

Dnmt1

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

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 or -

80°C. Avoid freeze / thaw cycle.

Storage buffer pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our

scientific support team who will be happy to help.

Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab19905 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	★★★★ ★ (4)	Use a concentration of 1 µg/ml. Detects a band of approximately 183 kDa (predicted molecular weight: 183 kDa).
ICC/IF	★★★★ <u>(1)</u>	Use a concentration of 1 µg/ml.
IP	★★★★ (2)	Use at an assay dependent concentration.
IHC-P	★ ★ ★ ☆ ☆ (4)	Use a concentration of 1 - 10 µg/ml.

Target

Function Methylates CpG residues. Preferentially methylates hemimethylated DNA. Associates with DNA

replication sites in S phase maintaining the methylation pattern in the newly synthesized strand, that is essential for epigenetic inheritance. Associates with chromatin during G2 and M phases to

maintain DNA methylation independently of replication. It is responsible for maintaining

methylation patterns established in development. DNA methylation is coordinated with methylation of histones. Mediates transcriptional repression by direct binding to HDAC2. In association with

DNMT3B and via the recruitment of CTCFL/BORIS, involved in activation of BAG1 gene expression by modulating dimethylation of promoter histone H3 at H3K4 and H3K9.

Tissue specificityUbiquitous; highly expressed in fetal tissues, heart, kidney, placenta, peripheral blood

mononuclear cells, and expressed at lower levels in spleen, lung, brain, small intestine, colon,

liver, and skeletal muscle. Isoform 2 is less expressed than isoform 1.

Sequence similarities Belongs to the C5-methyltransferase family.

Contains 2 BAH domains.

Contains 1 CXXC-type zinc finger.

Domain The N-terminal part is required for homodimerization and acts as a regulatory domain.

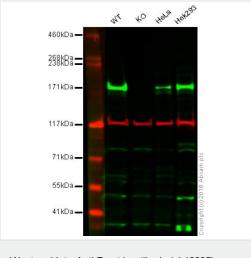
Post-translational

modifications

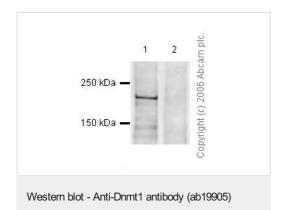
 $Sum oy lated; sum oy lation increases \ activity. \\$

Cellular localization Nucleus.

Images



Western blot - Anti-Dnmt1 antibody (ab19905)



Lane 2: DNMT1 knockout HAP1 whole cell lysate (20 µg)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)

Lane 3: HeLa whole cell lysate (20 µg)

Lane 4: Hek293 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab19905 observed at 170 kDa. Red - loading control, ab18058, observed at 130 kDa.

ab19905 was shown to recognize DNMT1 when DNMT1 knockout samples were used, along with additional cross-reactive bands. Wild-type and DNMT1 knockout samples were subjected to SDS-PAGE. Ab19905 and ab18058 (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 1 ug/ml and 1/10000 dilution respectively. Blots were developed with 800CW Goat anti Rabbit and 680CW Goat anti Mouse secondary antibodies at 1/10010 dilution for 1 hour at room temperature before imaging.

All lanes: Anti-Dnmt1 antibody (ab19905) at 1 µg/ml

Lane 1: HeLa nuclear lysate

Lane 2: HeLa nuclear lysate with Human Dnmt1 peptide

(ab21999) at 1 µg/ml

Lysates/proteins at 20 µg per lane.

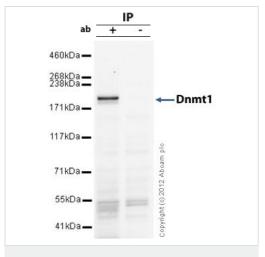
Secondary

All lanes: Goat polyclonal to Rabbit IgG (Alexa Fluor® 680) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 183 kDa Observed band size: 183 kDa

ab19905 specifically recognises Dnmt1 at 183 kDa in HeLa nuclear extracts (lane1), which is efficiently blocked using the immunising peptide (ab21999) (lane2).



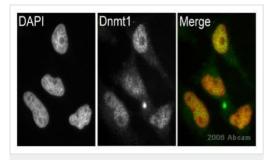
Immunoprecipitation - Anti-Dnmt1 antibody (ab19905)

Dnmt1 was immunoprecipitated using 0.5mg Hela whole cell extract, 5µg of Rabbit polyclonal to Dnmt1 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-). The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40μ I SDS loading buffer and incubated for 10min at 70° C; 10μ I of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab 19905.

Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (ab99697).

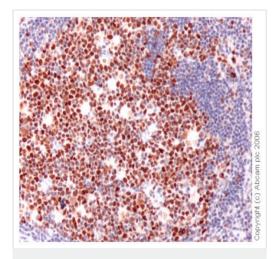
Band: 183kDa: Dnmt1.



Immunocytochemistry/ Immunofluorescence - Anti-Dnmt1 antibody (ab19905)

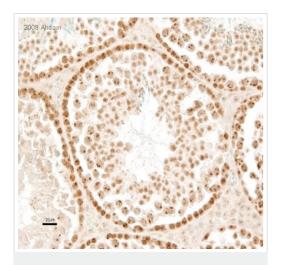
ab19905 at a 1/200 dilution staining paraformaldehyde fixed asynchronous HeLa cells by ICC/IF. The antibody was incubated with the cells for 30 minutes and then bound antibody was detected using a Cy3 conjugated goat anti-rabbit antibody. Nuclei were visualised using DAPI staining. Ab19905 gives a pattern that is predominantly enriched within nuclei.

This image is courtesy of an Abreview submitted by **Kirk McManus.**



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Dnmt1 antibody (ab19905)

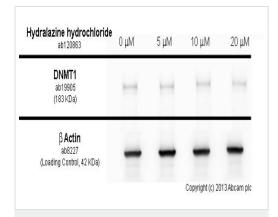
Staining of human tonsil tissue was performed using ab19905. Strong nuclear staining was observed in germinal center cells and scattered cells in the interfollicular area. T cells were weakly to moderately positive and endothelial cells were positive.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Dnmt1 antibody (ab19905)

This image is courtesy of an anonymous Abreview

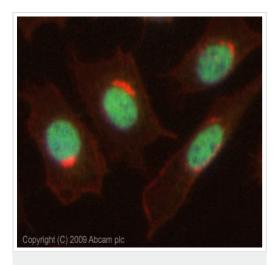
ab19905 (1/100) staining Dnmt1 in paraffin-embbeded mouse testis tissue sections. Tissue underwent fixation in formaldehyde, heat-mediated antigen retrieval in 10mM citrate buffer pH 6.0 and blocking (30 minutes, 8% milk). A biotinylated anti-rabbit lgG (H+L) antibody was used as the secondary. For further experimental details please refer to abreview.



Western blot - Anti-Dnmt1 antibody (ab19905)

Jurkat cells were incubated at 37° C for 5 days with vehicle control (0 μ M) and different concentrations of hydralazine hydrochloride (<u>ab120863</u>). Decreased expression of DNMT1 (ab19905) in Jurkat cells correlates with an increase in hydralazine hydrochloride concentration, as described in literature.

Whole cell lysates were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 20 μ g of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 5% BSA before being incubated with ab19905 at 1 μ g/ml and ab8227 at 1 μ g/ml overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP (ab97051) at 1/10000 dilution and visualised using ECL development solution.



Immunocytochemistry/ Immunofluorescence - Anti-Dnmt1 antibody (ab19905)

ICC/IF image of ab19905 stained HeLa cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab19905, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM. This antibody also gave a positive result in 1% PFA fixed (10 min) Hek293, HepG2 and MCF7 cells at 1µg/ml.

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