abcam

ab92314

Anti-Dnmt1 antibody [EPR3522]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.05% Sodium azide Constituents: 50% Tissue culture supernatant, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 0.1% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR3522
Purification technique	Affinity purification Protein A
Concentration	0.769 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/20
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000 - 1/10000
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/50 - 1/100
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/100 - 1/1000
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

ChIP

Not recommended

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

Target data

See full target information DNMT1

Function	DNA methyltransferase that methylates CpG residues (PubMed:17200670, PubMed:18754681, PubMed:21745816, PubMed:26070743). Preferentially methylates hemimethylated DNA (PubMed:21745816, PubMed:26070743). Associates with DNA replication sites in S phase maintaining the methylation pattern in the newly synthesized strand, that is essential for epigenetic inheritance (PubMed:17200670, PubMed:21745816). Associates with chromatin during G2 and M phases to maintain DNA methylation independently of replication (PubMed:21745816). It is responsible for maintaining methylation patterns established in development (PubMed:21745816). DNA methylation is coordinated with methylation of histones (PubMed:16357870). Mediates transcriptional repression by direct binding to HDAC2 (PubMed:10888872). 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 (PubMed:18413740). Probably forms a corepressor complex required for activated KRAS-mediated promoter hypermethylation and transcriptional silencing of tumor suppressor genes (TSGs) or other tumor-related genes in colorectal cancer (CRC) cells (PubMed:24623306). Also required to maintain a transcriptionally repressive state of genes in undifferentiated embryonic stem cells (ESCs) (PubMed:24623306). Associates at promoter regions of tumor suppressor genes (TSGs) leading to their gene silencing (PubMed:24623306).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Storage information	Stable for 12 months at -20°C

Notes

Species reactivity
Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

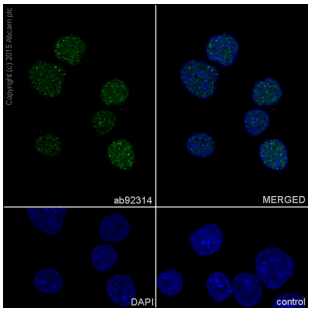
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

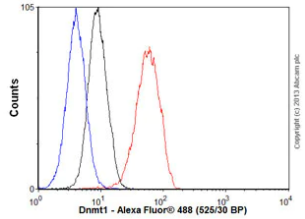
7 product images



Immunocytochemistry/ Immunofluorescence - Anti-Dnmt1 antibody [EPR3522] (ab92314)

Dnmt1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Dnmt1 antibody

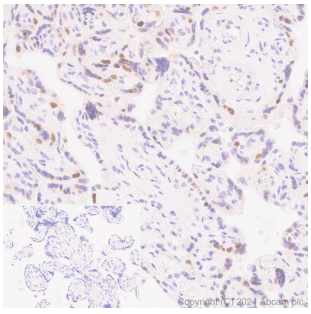
Immunofluorescence staining of Jurkat cells with purified ab92314 at a working dilution of 1/2000, counter-stained with DAPI. The secondary antibody was an Alexa Fluor® 488 conjugated goat anti-rabbit (ab150077), used at a dilution of 1/1000. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative control is shown in bottom right hand panel - for the negative control, PBS was used instead of the primary antibody.



Flow Cytometry (Intracellular) - Anti-Dnmt1 antibody [EPR3522] (ab92314)

Dnmt1 Flow Cytometry (Intracellular) staining using rabbit Anti-Dnmt1 antibody

Overlay histogram showing HeLa cells stained with ab92314 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab92314, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Dnmt1 antibody [EPR3522] (ab92314)

Dnmt1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human placenta using rabbit Anti-Dnmt1 antibody

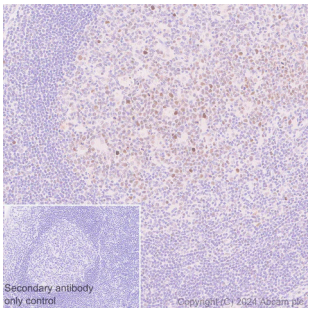
Immunohistochemical analysis of paraffin-embedded Human placenta tissue labeling Dnmt1 with ab92314 at 1/200 (3.55 µg/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on human placenta. The section was incubated with ab92314 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Dnmt1 antibody [EPR3522] (ab92314)

Dnmt1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human tonsil using rabbit Anti-Dnmt1 antibody

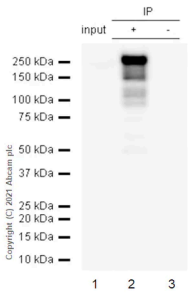
Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling Dnmt1 with ab92314 at 1/200 (3.55 µg/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on human tonsil. The section was incubated with ab92314 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Immunoprecipitation - Anti-Dnmt1 antibody [EPR3522] (ab92314)

Dnmt1 was immunoprecipitated from 0.35 mg Hut-78 (Human Sezary syndrome cutaneous T lymphocyte) whole cell lysate 10 µg with 92314 at 1/50 dilution (2µg). VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.
Lane 1: Hut-78 (Human Sezary syndrome cutaneous T lymphocyte) whole cell lysate 10 µg

Lane 2: ab92314 IP in Hut-78 whole cell lysate

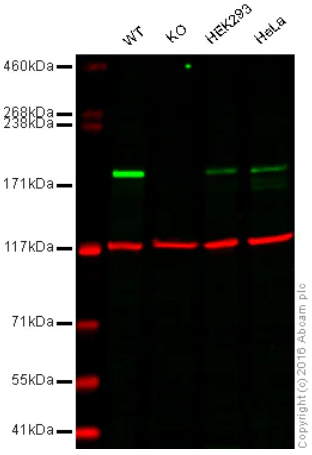
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab92314 in Hut-78 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

All lanes:
Immunoprecipitation - Anti-Dnmt1 antibody [EPR3522] (ab92314)

Predicted band size: 183 kDa

Observed band size: 183 kDa



Western blot - Anti-Dnmt1 antibody [EPR3522] (ab92314)

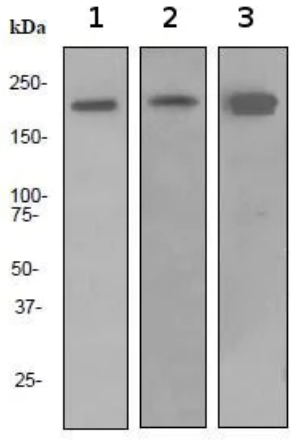
Dnmt1 Western blot staining using rabbit Anti-Dnmt1 antibody

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: DNMT1 knockout HAP1 whole cell lysate (20 µg)
Lane 3: HEK293 whole cell lysate (20 µg)
Lane 4: HeLa whole cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab92314 observed at 170 kDa. Red - loading control, ab18058, observed at 130 kDa.

ab92314 was shown to specifically react with DNMT1 when DNMT1 knockout samples were used. Wild-type and DNMT1 knockout samples were subjected to SDS-PAGE. ab92314 and ab18058 (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 1000 dilution and 1/10000 dilution respectively. Blots were developed with 800CW Goat anti Rabbit and 680CW Goat anti Mouse secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Dnmt1 antibody [EPR3522] (ab92314)

Predicted band size: 183 kDa



Western blot - Anti-Dnmt1 antibody [EPR3522] (ab92314)

All lanes:
Western blot - Anti-Dnmt1 antibody [EPR3522] (ab92314) at 1/1000 dilution

Lane 1:
HuT-78 cell lysate at 10 µg

Lane 2:
Jurkat cell lysate at 10 µg

Lane 3:
293T cell lysate at 10 µg

Secondary

All lanes:
HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 183 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.