# abcam

## Product datasheet

## Anti-Dnmt1 antibody [EPR3522] ab92314



Recombinant RabMAb

## 12 References 6 Images

#### Overview

**Product name** Anti-Dnmt1 antibody [EPR3522]

Rabbit monoclonal [EPR3522] to Dnmt1 **Description** 

**Host species** Rabbit

**Tested applications** Suitable for: Flow Cyt (Intra), WB, IP, ICC/IF

Unsuitable for: ChIP or IHC-P

Reacts with: Human Species reactivity

Synthetic peptide within Human Dnmt1 aa 1600-1700 (C terminal). The exact sequence is **Immunogen** 

proprietary.

Positive control WB: HuT-78, Jurkat or 293T lysate. ICC: Jurkat cells. Flow Cyt: HeLa cells IP: Hut-78.

**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.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

### **Properties**

**Form** 

Storage instructions Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.

Storage buffer pH: 7.20

Preservative: 0.05% Sodium azide

Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue

culture supernatant

Protein A purified **Purity** 

Clonality Monoclonal
Clone number EPR3522
Isotype IqG

#### **Applications**

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab92314 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)		1/100 - 1/1000. <b>ab172730</b> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB		1/1000 - 1/10000. Predicted molecular weight: 183 kDa.
IP		1/20.
ICC/IF		1/50 - 1/100.

**Application notes** Is unsuitable for ChIP or IHC-P.

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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  $\frac{1}{2}$ 

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 specificity** Ubiquitous; 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.

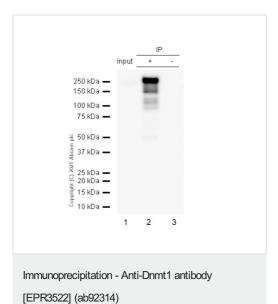
Post-translational

modifications

Sumoylated; sumoylation increases activity.

Cellular localization Nucleus.

## **Images**



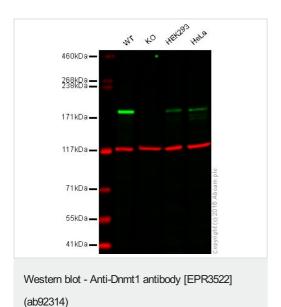
Dnmt1 was immunoprecipitated from 0.35 mg Hut-78 (Human Sezary syndrome cutaneous T lymphocyte) whole cell lysate 10  $\mu$ g with 92314 at 1/50 dilution (2 $\mu$ g). VeriBlot for IP Detection Reagent (HRP)(<u>ab131366</u>) was used at 1/5000 dilution.

**Lane 1**: Hut-78 (Human Sezary syndrome cutaneous T lymphocyte) whole cell lysate 10  $\mu g$ 

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

**Lane 3**: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab92314 in Hut-78 whole cell lysate

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



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

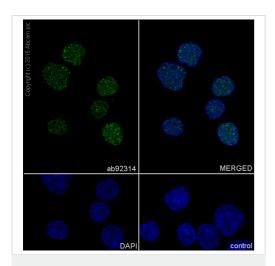
Lane 2: DNMT1 knockout HAP1 whole cell lysate (20 µg)

Lane 3: HEK293 whole cell lysate (20 µg)

Lane 4: HeLa whole cell lysate (20 µg)

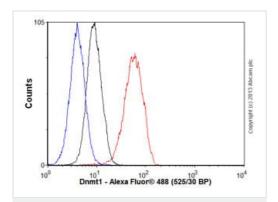
**Lanes 1 - 4:** Merged signal (red and green). Green - ab92314 observed at 170 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

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 <u>ab18058</u> (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.



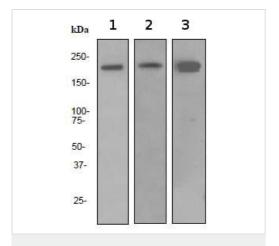
Immunocytochemistry/ Immunofluorescence - Anti-Dnmt1 antibody [EPR3522] (ab92314)

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<sup>®</sup> 488 conjugated goat antirabbit (<u>ab150077</u>), 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)

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<sup>®</sup> 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<sup>6</sup> 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.



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

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

Lane 1 : HuT-78 cell lysate
Lane 2 : Jurkat cell lysate
Lane 3 : 293T cell lysate

Lysates/proteins at 10 µg per lane.

#### Secondary

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

Predicted band size: 183 kDa



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