

Anti-Dnmt1 (1-248) antibody, affinity-purified (rabbit polyclonal)

70-201 50 ug

Shipping and Storage temperatures: Ship at 4° C or -20° C and store at -20° C. Do not freeze,

Reactivity: Mouse and human. Not tested for other species

Vakidated by western blot with Dnmt1 KO cells and recombinant GST Dnmt1 (1-248) protein.

Immunogen: Highly purified recombinant mouse Dnmt1 (amino acids 1-248), soluble form

Applications

1) Western blotting (0.2~1 ug/ml)

- 2) Immunoprecipitation. Especially good for native form of Dnmt1 and useful for analysis of the protein complex containing Dnmt1. One ug of the antibody can precipitates 0.5~1.0 ug of Dnmt1.
- 3) Indirect immuno-fluorescence staining. Excellent. Low background image will be obtained by using the antibody at 5,000 fold dilution.
- 4) Chromatin Immuno-Precipitation (Assay dependent)

Purity: Affinity-purified with the recombinant Dnmt1,

Form: 1 mg/ml in PBS, 50% glycerol.

Background: Dnmt1 (DNA (cytosine-5-)-methyltransferase 1) has a role in the establishment and regulation of tissue-specific patterns of methylated cytosine residues (epigenetics). Hypermethylation in promoter regions are associated with repression of the genes. Aberrant methylation patterns are associated with certain human tumors and developmental abnormalities.

Data Link: UniProtKB/Swiss-Prot P13864 (DNMT1_MOUSE)

1 2

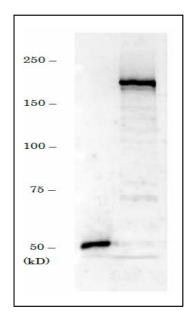


Fig.1 Western blot of endogenous Dnmt1 in crude extract of NIH3T3 cells.

Samples.

- 1. Immunogen, GST-Dnmt3 (1-248) fusion protein
- 2. Whole cell extract of NIH3T3 cells (20 μ g)

The anti-Dnmt1 antibody was used at 1 μ g/ml and 2nd atnbody, goat anti-rabbit IgG antibody conjugated with HRP was used at 1/10,000 dilution.



WT KO



Fig.2 Validation of the anti-Dnmt1 antibody by western blot, using Dnmt1 KO cell extract.

Lane 1: Mouse ES cell line J1 (Dnmt1 +/+)

Lane 2: Mouse ES cell line 36 (Dnmt1 -/-)

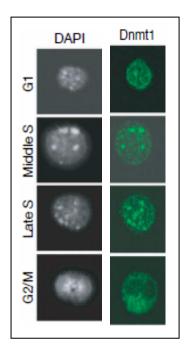
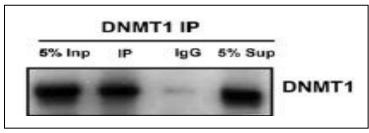


Fig.3 Immunofluorescence staining of Dnmt1 during cell cycle progression.

Embryonic stem cells (E14) during cell-cycle progression were reacted with anti-Dnmt1 antibody as primary antibody at 1/5,000 and with goat anti-rabbit IgG conjugated with Alexa Fluora 555 at 1/2,000. Nuclei were stained with DAPI.



182 kDa

Fig.4 Immunoprecipitation of Dnmt1 from mouse MEL cells.

The anti-Dnmt1 antibody attached to protein A conjugated magnetic beads was used. Non-immunized IgG was used as negative control.

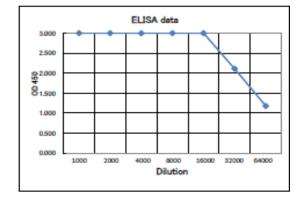


Fig.5. Titration of antibody reactivity of anti-Pertussis antiserum by direct ELISA Plate was coated with 100 ng of GST-Dnmt1 (1-248) per well and 100 µl of the antiserum at the indicated dilution was added to each well and incubated. After washing, goat anti-rabbit-IgG conjugated with HRP was added as 2nd antibody. Color was developed with TMB as substrate.

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References: This antibody has been used in the following publications.

- 1.Negishi M *et al.* 'Bmi1 cooperates with Dnmt1-associated protein 1 in gene silencing." <u>Biochem Biophys Res Commun.</u> 2007 Feb 23:353(4):992-8. PMID:17214966. **WB (mouse)**
- 2 Sharif J *et al*. The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA" *Nature* **450**:908-912 (2007) PMID: <u>17994007</u>. **WB, IP, IF (mouse, human)**
- 3. Papageorgiou DN *et al*. Distinct and overlapping DNMT1 interactions with multiple transcription factors in erythroid cells: Evidence for co-repressor functions. <u>Biochim Biophys</u>
 Acta. 1859 (12):1515-1526 (2016). PMID: 27693117. WB, IP, ChIP (human, mouse)
- 4.Maenohara S et al. 'Role of UHRF1 in de novo DNA methylation in oocytes and maintenance methylation in preimplantation embryos." <u>PLoS Genet.</u> 2017 Oct 4;13(10):e1007042 PMID: 28976982. **IF (mouse)**

Related products: #70-205 Anti-Dnmt3b antibody, affinity-purified (rabbit polyclonal)

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