

## Anti-Dnmt3b antibody, affinity-purified (rabbit polyclonal), CHIP grade

Catalog # 70-206 100 µg

Dnmt3b (DNA (cytosine-5-)-methyltransferase 3b) has a role in the establishment and regulation of tissue-specific patterns of methylated cytosine residues (epigenetics). Dnmt3b is thought to function in *de novo* methylation, rather than maintenance methylation. The protein localizes primarily to the nucleus and its expressions are developmentally regulated. Mutations in this gene cause the immunodeficiency-centromeric instability-facial anomalies (ICF) syndrome.

This antibody was prepared and characterized by Prof. S. Tajima of Osaka Univ. and used in Ref. 2~4.

### Applications

- 1) Western blot 1,000-fold dilution
- 2) Immunoprecipitation 1,000-fold dilution, useful for ChiP assays
- 3) Indirect immunofluorescence staining 1,000-fold dilution
- 4) Immunohistochemistry (1,000-fold)

### Specification

Immunogen: Highly purified recombinant mouse Dnmt3b (amino acids 1-181) (accession no. [AF068626](#))

Antibody: Affinity-purified with the recombinant Dnmt3b

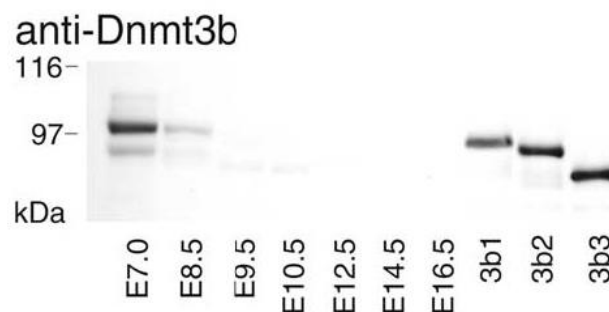
Reactivity: Reacts with mouse Dnmt3b. It may also react with other rodent homologs due the sequence homology, but it does not react with human homolog due to the sequence difference.

Form: 1 mg/ml in PBS, 50% glycerol, 0.05% sodium azide (and trace of ammonium sulfate)

Storage: ship 4°C and store at -20°C or long term storage -80°C

### Data Link

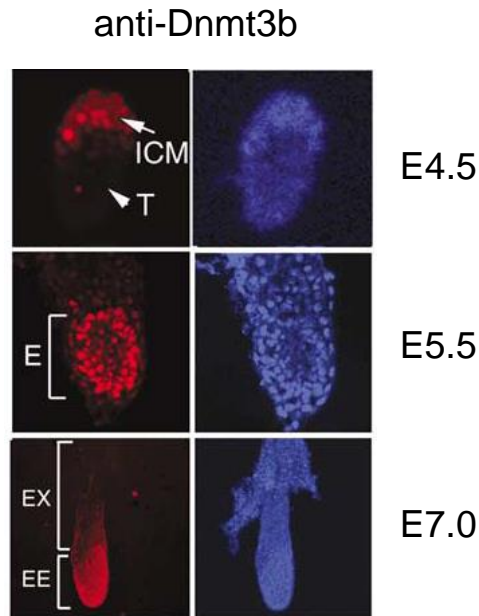
UniProtKB/Swiss-Prot [O88509](#) (DNM3B\_MOUSE)



**Fig. 1 Western blot of Dnmt3b.**

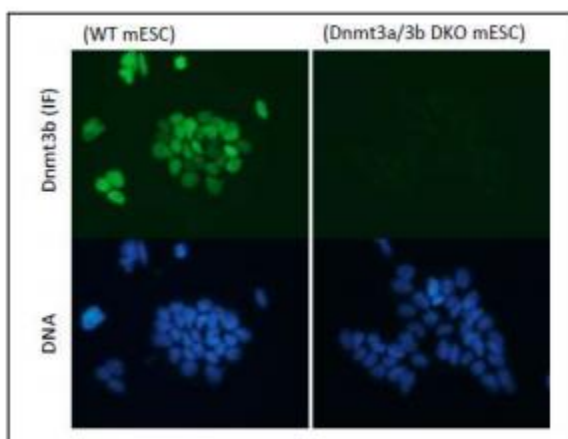
The amounts of Dnmt3b in mouse embryos at the stages of E7.0-E16.5 were examined by Western blot. The embryos were solubilized by sonication or homogenization in the presence of 0.1% SDS. The dissected embryo (10 µg protein) at each stage was subjected to Western blot with this antibody. Dnmt3b was highly expressed in E7.0 embryo but decreased thereafter and was below the detection level after E10.5. cDNAs of Dnmt3b isoforms (3b1, 3b2, and 3b3) were transiently expressed in 293T cells and these isoforms were also detectable with this antibody.

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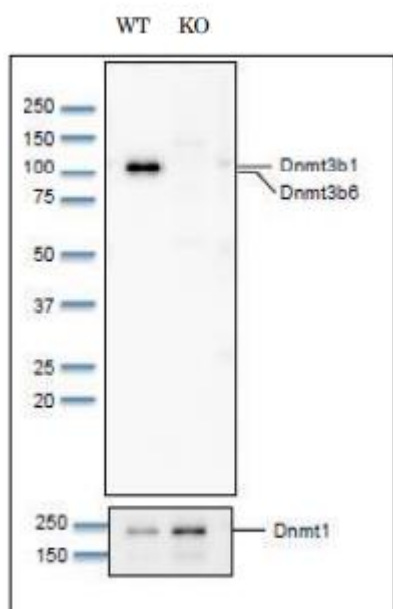


**Fig. 2 Expression of Dnmt3b in mouse embryos was examined by immunofluorescence staining.**

Mouse embryos at E4.5, 5.5 and 7.0 were fixed in cold acetone and stained with this antibody or DAPI (blue). The antibody was detected with anti-rabbit IgG conjugated with ALEXA568 (red). The ICM (the inner cell mass) and trophoblast (T) are indicated by arrows and arrowheads, respectively. The epiblast (E), and the embryonic ectoderm (EE) and extraembryonic region (EX) are indicated by square brackets. Dnmt3b existed at E4.5-7.0, in ICM at E4.5, the epiblast at E5.5, and the embryonic ectoderm at E7.0.



**Fig.3 Immunofluorescent staining of Dnmt3b in wild-type and Dnmt3a/3b knockout mouse embryonic stem cells.** Cells were grown in GMEM 10% FCS+LIF in gelatinized 48-well plate. Fixed with 4% PFA/PBS for 30 min at 4°C. Anti-Dnmt3b antibody was used at 1/5,000. DNA was stained with DAPI



**Fig.4 Western blot of Dnmt3b in wild-type and Dnmt3b/3a knockout mouse embryonic stem cells.** Left lane; Extract of wild-type ES cells (~10<sup>4</sup> cells) Right lane; Extract of Dnmt3a/3b KO cells. (~3 x10<sup>4</sup> cells) 8% SDS-PAGE Blotted onto PVDF. Blocked with 5% skim milk. The antibody was used at 1/3,000. Image was taken with Chemi-Lumi One Super LAS4000, 10 min exposure. As a control, western blot was done with Dnmt1 antibody reprobred.

**References:** This antibody was used for Western blot and immunohistochemistry in Ref. 2-4.

1. Rhee I *et al* "DNMT1 and DNMT3b cooperate to silence genes in human cancer cells" *Nature* 416: 552-6 (2002) PMID: [11932749](#)
  - 2 Aoki A *et al* "Enzymatic properties of *de novo*-type mouse DNA (cytosine-5) methyltransferases" *Nucleic Acids Research* 29: 3506-3512 (2001) PMID: [11522819](#)
  3. Watanabe D *et al* "Stage- and cell-specific expression of Dnmt3a and Dnmt3b during embryogenesis" *Mechanisms of Development* 118: 187-190 (2002) PMID: [12351185](#)
  4. Sakai Y *et al* "Co-expression of *de novo* DNA methyltransferases Dnmt3a2 and Dnmt3L in gonocytes of mouse embryos" *Gene Expression Patterns* 5: 231-237 (2004) PMID: [15567719](#)
- The data of Western blot and immunofluorescence staining obtained by using this antibody are shown in the next page.
5. Suetake I *et al*. "Distinct enzymatic properties of recombinant mouse DNA methyltransferases Dnmt3a and Dnmt3b." *J Biochem.* 2003 Jun;133(6):737-44. PMID: 12869530
  6. Suetake I *et al*. "DNMT3L stimulates the DNA methylation activity of Dnmt3a and Dnmt3b through a direct interaction." *J Biol Chem.* 2004 Jun 25;279(26):27816-23. PMID:15105426 WB, IP (mouse)
  7. Watanabe D. *et al*. "Expression of Dnmt3b in mouse hematopoietic progenitor cells and spermatogonia at specific stages. *Gene Expr Patterns.* 2004 Nov;5(1):43-9. PMID:15533817 IHC: mouse
  8. Sakai Y *et al*. "Co-expression of *de novo* DNA methyltransferases Dnmt3a2 and Dnmt3L in gonocytes of mouse embryos." *Gene Expr Patterns.* 2004 Dec;5(2):231-7. PMID:15567719 WB, KHC (mouse) 8.
  9. Watanabe D *et al*. "Transition of mouse *de novo* methyltransferases expression from Dnmt3b to Dnmt3a during neural progenitor cell development". *Neuroscience.* 2006 Oct 27;142(3):727-37. PMID:16973295. IHC (mouse)
  10. Hirasawa R *et al*. "Maternal and zygotic Dnmt1 are necessary and sufficient for the maintenance of

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DNA methylation imprints during preimplantation development." Genes Dev. 2008 Jun 15;22(12):1607-16.  
IF (mouse)

Related product: #70-201 Anti-Dnmt1 (1-248) antibody, affinity-purified (rabbit polyclonal)  
#70-203 Anti-Dnmt1 (1037-1086) antibody, affinity-purified (rabbit polyclonal)