

Anti-Dnmt3b antibody, rabbit polyclonal, CHIP grade, KO-Validated

70-206 100 μ l

Shipping and Storage: Shipped at 4°C or -20°C and store at -20°C.

Immunogen: Recombinant mouse Dnmt3b (amino acids 1-181)

Form: ~0.3 mg/ml in PBS⁻ with 50% glycerol. Azide⁻ and carrier-free

Purity: Affinity-purified with immunogen.

Reactivity: Reacts with mouse Dnmt3b isoforms (3b1, 3b2, 3b3 and 3b6). Does not react with Dnmt3a due to the sequence difference in the N-terminal regions used as the immunogen.

Applications

- 1) Western blotting (1/3,000)
- 2) Immunoprecipitation (1/1,000),
- 3) ChiP assays (assay dependent)
- 4) Indirect immuno-fluorescence staining (1/5,000)

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

Data Link UniProtKB/Swiss-Prot [O88509](#) (DNM3B_MOUSE)

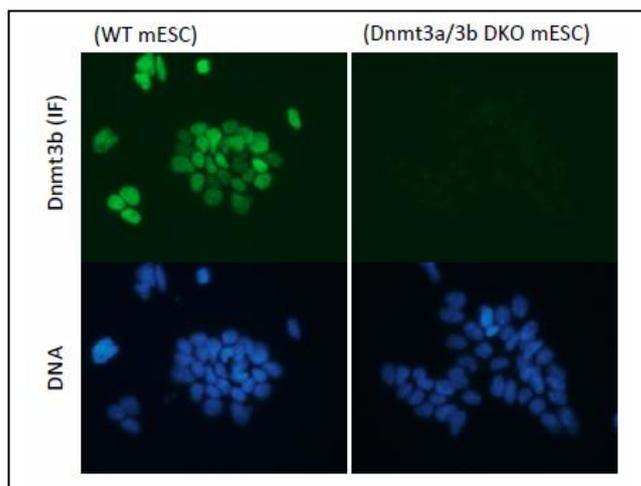


Fig.1 Immunofluorescent staining of Dnmt3b in wild-type and Dnmt3b/3a knockout mouse embryonic stem cells.

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.

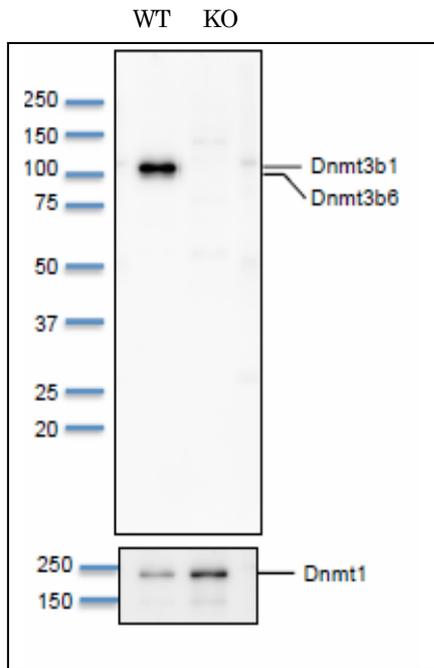


Fig.2 Western blot of Dnmt3b in wild-type and Dnmt3b/3a knockout mouse embryonic stem cells.

Left lane; Extract of wild-type ES cells. (~10⁴ cells)
Right lane; Extract of Dnmt3a/3b KO cells. (~3 x10⁴ 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 reprobed.

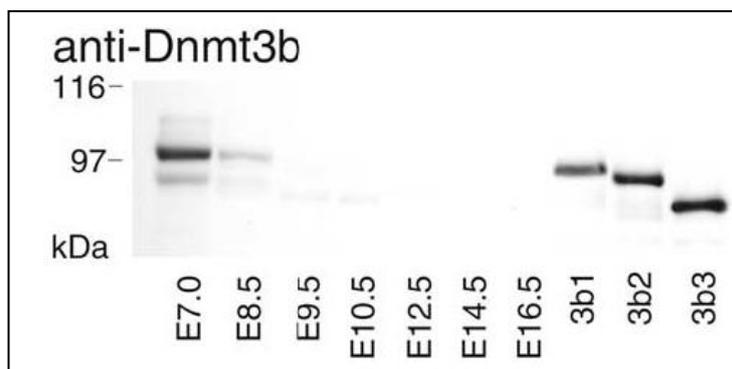


Fig.3 Western blotting of Dnmt3b at different stage of embryo.

The amounts of Dnmt3b in mouse embryos at the stages of E7.0-E16.5 were examined by Western blotting. The embryos were solubilized by sonication or homogenization in the presence of 0.1% SDS. The dissected embryo (10 ug protein) at each stage was subjected to Western blotting 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.

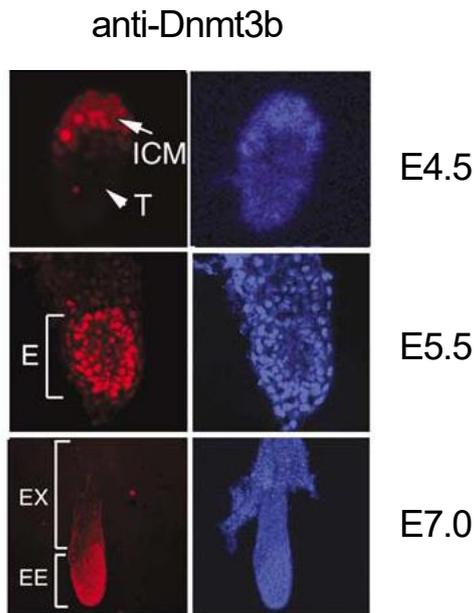


Fig. 4 Expression of Dnmt3b in mouse embryo was examined by immuno-fluorescence staining.

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.

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

1. Aoki A *et al* "Enzymatic properties of *de novo*-type mouse DNA (cytosine-5) methyltransferases" *Nucleic Acids Research* **29**: 3506-3512 (2001) PMID: [11522819](#) **WB (mouse)**
2. Watanabe D *et al* "Stage- and cell-specific expression of Dnmt3a and Dnmt3b during embryogenesis" *Mechanisms of Development* **118**: 187-190 (2002) PMID: [12351185](#) **WB, IF, IHC-F (mouse)**
3. 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)**
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](#) **WB, IHC-F (mouse)**
5. Hirasawa R *et al*. "Maternal and zygotic Dnmt1 are necessary and sufficient for the maintenance of DNA methylation imprints during preimplantation development." *Genes Dev.* 2008 Jun 15;22(12):1607-16. PMID: [18559477](#) **IF (mouse)**

Related product: #70-201 Anti-Dnmt1 (1-248) antibody, rabbit polyclonal, ChIP grade

Please note: All products are FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR MILITARY USE.