#5032 Store at -20°C

DNMT1 (D63A6) XP® Rabbit mAb

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

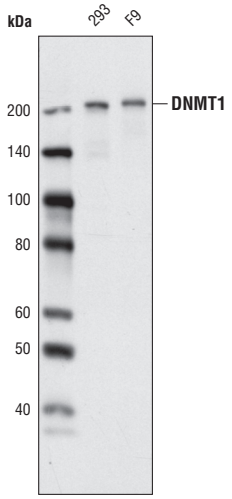
Entrez-Gene ID #1786
UniProt ID #P26358

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IF-IC, F Endogenous	H, M, R, Mk, (B, Hm, Dg, Guinea Pig, Hr)	200 kDa	Rabbit IgG**

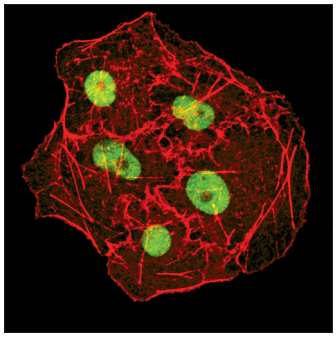
Background: Methylation of DNA at cytosine residues in mammalian cells is a heritable, epigenetic modification that is critical for proper regulation of gene expression, genomic imprinting and development (1,2). Three families of mammalian DNA methyltransferases have been identified: DNMT1, DNMT2 and DNMT3 (1,2). DNMT1 is constitutively expressed in proliferating cells and functions as a maintenance methyltransferase, transferring proper methylation patterns to newly synthesized DNA during replication. DNMT3A and DNMT3B are strongly expressed in embryonic stem cells with reduced expression in adult somatic tissues. DNMT3A and DNMT3B function as *de novo* methyltransferases that methylate previously unmethylated regions of DNA. DNMT2 is expressed at low levels in adult somatic tissues and its inactivation affects neither *de novo* nor maintenance DNA methylation. DNMT1, DNMT3A and DNMT3B together form a protein complex that interacts with histone deacetylases (HDAC1, HDAC2, Sin3A), transcriptional repressor proteins (RB, TAZ-1) and heterochromatin proteins (HP1, SUV39H1), to maintain proper levels of DNA methylation and facilitate gene silencing (3-8). Improper DNA methylation contributes to diseased states such as cancer (1,2). Hypermethylation of promoter CpG islands within tumor suppressor genes correlates with gene silencing and the development of cancer. In addition, hypomethylation of bulk genomic DNA correlates with and may contribute to the onset of cancer. DNMT1, DNMT3A and DNMT3B are overexpressed in many cancers, including acute and chronic myelogenous leukemias, in addition to colon, breast and stomach carcinomas (9-12).

Specificity/Sensitivity: DNMT1 (D63A6) XP® Rabbit mAb detects endogenous levels of total DNMT1 protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to the sequence of human DNMT1 protein.



Western blot analysis of extracts from 293 and F9 cells using DNMT1 (D63A6) XP® Rabbit mAb.



Confocal immunofluorescent analysis of COS cells using DNMT1 (D63A6) XP® Rabbit mAb (green). Actin filaments were labeled using DyLight™ 554 Phalloidin #13054 (red).

Storage: Supplied in 10 mM HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. *Do not aliquot the antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western Blotting	1:1000
Immunofluorescence (IF-IC)	1:100
Flow Cytometry	1:50

For application specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

(1) Hermann, A. et al. (2004) *Cell. Mol. Life Sci.* 61, 2571-2587.

(2) Turek-Plewa, J. and Jagodzinski, P.P. (2005) *Cell. Mol. Biol. Lett.* 10, 631-647.

(3) Kim, G.D. et al. (2002) *EMBO J.* 21, 4183-4195.

(4) Fuks, F. et al. (2001) *EMBO J.* 20, 2536-2544.

(5) Geiman, T.M. et al. (2004) *Biochem. Biophys. Res. Commun.* 318, 544-555.

(6) Rountree, M.R. et al. (2000) *Nat. Genet.* 25, 269-277.

(7) Pradhan, S. and Kim, G.D. (2002) *EMBO J.* 21, 779-788.

(8) Fuks, F. et al. (2003) *Nucleic Acids Res.* 31, 2305-2312.

(9) Mizuno, S. et al. (2001) *Blood* 97, 1172-1179.

(10) Robertson, K.D. et al. (1999) *Nucleic Acids Res.* 27, 2291-2298.

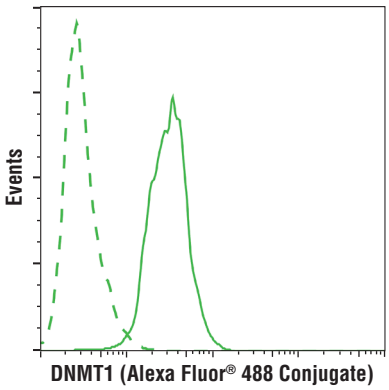
(11) Xie, S. et al. (1999) *Gene* 236, 87-95.

(12) Kanai, Y. et al. (2001) *Int. J. Cancer* 91, 205-212.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Flow cytometric analysis of COS-7 cells using DNMT1 (D63A6) XP® Rabbit mAb (solid line) compared to concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (dashed line). Anti-rabbit IgG (H+L) F(ab')$_2$ Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.