Revision 7

Store at -20C

#5032

DNMT1 (D63A6) XP® Rabbit mAb

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, IHC-P, IF-IC, FC-FP, ChIP	H M R Mk	Endogenous	200	Rabbit IgG	#P26358	1786

Product Usage Information

For optimal ChIP results, use 10 µl of antibody and 10 µg of chromatin (approximately 4 x 10^6 cells) per IP. This antibody has been validated using SimpleChIP® Enzymatic Chromatin IP Kits.

Application	Dilution
Western Blotting	1:1000
Immunohistochemistry (Paraffin)	1:50 - 1:200
Immunofluorescence (Immunocytochemistry)	1:100
Flow Cytometry (Fixed/Permeabilized)	1:50 - 1:200
Chromatin IP	1:50

Storage

Supplied in 10 mM sodium 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.

For a carrier free (BSA and azide free) version of this product see product #28754.

Specificity/Sensitivity

DNMT1 (D63A6) XP® Rabbit mAb detects endogenous levels of total DNMT1 protein. Species reactivity for IHC-P is human only.

Species predicted to react based on 100% sequence homology

Hamster, Bovine, Dog, Horse, Guinea Pig

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Leu985 of human DNMT1 protein.

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).

Background References

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4. Fuks, F. et al. (2001) *EMBO J.* 20, 2536-44.
5. Geiman, T.M. et al. (2004) *Biochem. Biophys. Res. Commun.* 318, 544-55.
6. Rountree, M.R. et al. (2000) *Nat. Genet.* 25, 269-77.
7. Pradhan, S. and Kim, G.D. (2002) *EMBO J.* 21, 779-88.
8. Fuks, F. et al. (2003) *Nucleic Acids Res.* 31, 2305-12.
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10. Robertson, K.D. et al. (1999) *Nucleic Acids Res.* 27, 2291-8.

11. Xie, S. et al. (1999) *Gene* 236, 87-95.
12. Kanai, Y. et al. (2001) *Int. J. Cancer* 91, 205-12.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

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

Applications Key

W: Western Blotting **IHC-P:** Immunohistochemistry (Paraffin) **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized) **ChIP:** Chromatin IP

Cross-Reactivity Key

H: Human **M:** Mouse **R:** Rat **Mk:** Monkey

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Limited Uses