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DNMT3B (D7070) Rabbit mAb

#67259

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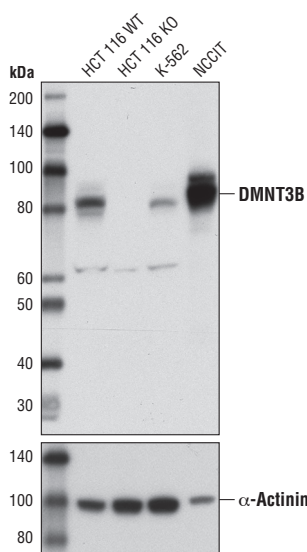
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Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IF-IC Endogenous	H	96 kDa	Rabbit IgG**

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 over-expressed in many cancers, including acute and chronic myelogenous leukemias, in addition to colon, breast and stomach carcinomas (9-12).

Specificity/Sensitivity: DNMT3B (D7070) Rabbit mAb recognizes endogenous levels of total DNMT3B protein. This antibody also detects a non-specific protein of approximately 65 kDa in multiple cell lines. Based on sequence homology, this antibody should recognize all isoforms of DNMT3B. This antibody shows low sensitivity in IF-IC, where it only detects DNMT3B in high expressing cells. However, this clone detects DNMT3B in both high and low expressing cells by western blot.

Source/Purification: Monoclonal antibody is produced by immunizing animals with recombinant protein surrounding Ala395 of human DNMT3B protein.



Western blot analysis of extracts from HCT 116 DNMT3B wild-type (HCT 116 WT), HCT 116 DNMT3B knockout (HCT 116 KO), K-562, and NCCIT cells, using DNMT3B (D7070) Rabbit mAb (upper) or α -Actinin (D6F6) XP[®] Rabbit mAb #6487 (lower).

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μ g/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
Immunoprecipitation	1:50
Immunofluorescence (IF-IC)	1:1600
IF Protocol:	Special Protocol Required

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Background References:

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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.

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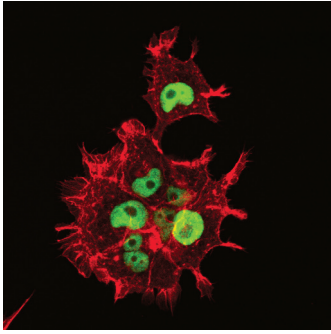
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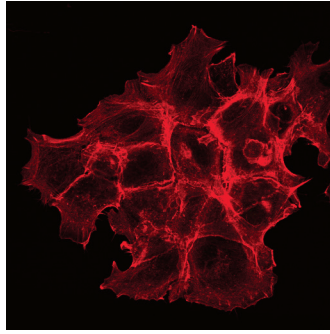
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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken 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.

NCCIT



HEK 293



Confocal immunofluorescent analysis of NCCIT (left) and HEK 293 (right) cells using DNMT3B (D7070) Rabbit mAb (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). HEK 293 cells express very low levels of DNMT3B protein.

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