

DESCRIPTION

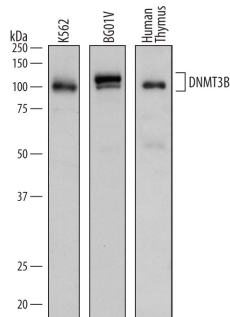
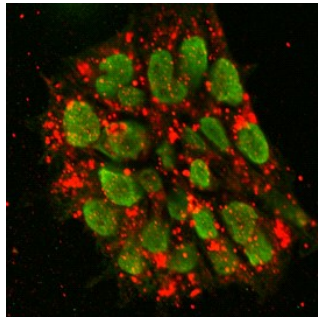
Species Reactivity	Human
Specificity	Detects human DNMT3B in direct ELISAs and Western blots. In direct ELISAs, less than 1% cross-reactivity with recombinant human DNMT3A is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human DNMT3B Ser145-Pro355 Accession # Q9UBC3
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	0.2 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below

DATA

<p>Western Blot</p>  <p>Detection of Human DNMT3B by Western Blot. Western blot shows lysates of K562 human chronic myelogenous leukemia cell line, BG01V human embryonic stem cells, and human thymus tissue. PVDF membrane was probed with 0.2 µg/mL of Sheep Anti-Human DNMT3B Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7646) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). Specific bands were detected for DNMT3B at approximately 100-110 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunocytochemistry</p>  <p>DNMT3B in BG01V Human Embryonic Stem Cells. DNMT3B and SSEA-4 were detected in immersion fixed BG01V human embryonic stem cells. DNMT3B was detected using Sheep Anti-Human DNMT3B Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7646) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 493-conjugated Anti-Sheep IgG Secondary Antibody (green; Catalog # NL012). SSEA-4 was detected using Mouse Anti-Human/Mouse SSEA-4 Monoclonal Antibody (Catalog # MAB1435) and stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007). Specific staining of DNMT3B was localized to nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.</p>
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PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

DNMT3B (DNA methyltransferase 3B; also DNA MTase HsallIB) is a 95-110 kDa member of the C5-methyltransferase family of enzymes. It is ubiquitously expressed in the embryo, and found associated with nuclear pericentric heterochromatin. Pericentric heterochromatin is chromatin composed of satellite repeats that lie adjacent to the centromere. DNA (cytosine-guanine dinucleotides) associated with this area undergoes methylation, contributing to gene repression and proper chromosome segregation. Human DNMT3B is 853 amino acids (aa) in length. It contains a PWWP (Pro-Trp-Trp-Pro) domain that binds DNA (aa 225-283), a ADD (ATRX, DNMT3, DNMT3L) domain that contains a Zn-finger and binds to an unmethylated histone H3 tail (aa 423-555), and a class I AdoMet-MTase catalytic region that uses S-adenosyl-L-homocysteine as a substrate for methyl transfer (aa 577-847). There are at least five isoform variants. All show a deletion of aa 365-375. In addition, a second contains an alternative start site 12 aa upstream of the standard site, a third shows a deletion of aa 745-807, a fourth contains a Ser substitution for aa 744-853, while a fifth possesses a 45 aa substitution for aa 768-853. Over aa 1-355, human DNMT3B shares 73% aa sequence identity with mouse DNMT3B.