

## DESCRIPTION

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

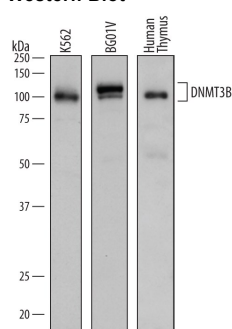
## APPLICATIONS

**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.

	<b>Recommended Concentration</b>	<b>Sample</b>
<b>Western Blot</b>	0.2 µg/mL	See Below
<b>Immunocytochemistry</b>	5-15 µg/mL	See Below
<b>Simple Western</b>	10 µg/mL	K562 human chronic myelogenous leukemia cell line
<b>Knockout Validated</b>	DNMT3B is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in DNMT3B knockout HeLa cell line.	

## DATA

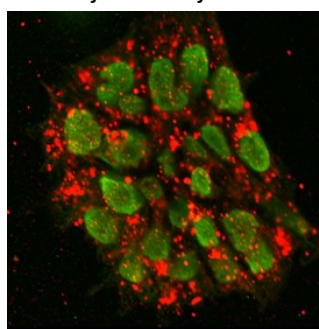
### Western Blot



#### 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 # 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.

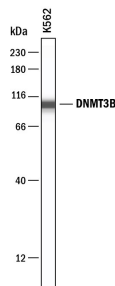
### Immunocytochemistry



#### 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 # Catalog # NL012). SSEA-4 was detected using Mouse Anti-Human/Mouse SSEA-4 Monoclonal Antibody (Catalog # Catalog # MAB1435) and stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # Catalog # NL007). Specific staining of DNMT3B was localized to nuclei. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

### Simple Western

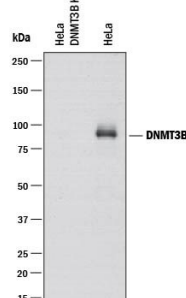


#### Detection of Human DNMT3B by Simple Western™.

Simple Western lane view shows lysates of K562 human chronic myelogenous leukemia cell line, loaded at 0.2 mg/mL. A specific band was detected for DNMT3B at approximately 102 kDa (as indicated) using 10 µg/mL of Sheep Anti-Human DNMT3B Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7646). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



### Knockout Validated



#### Western Blot Shows Human DNMT3B Specificity by Using Knockout Cell Line.

Western blot shows lysates of DNMT3B knockout HeLa cell line (KO) and HeLa human cervical epithelial carcinoma parental cell line. PVDF membrane was probed with 0.5 µg/mL of Sheep Anti-Human DNMT3B Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7646) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # Catalog # HAF016). A specific band was detected for DNMT3B at approximately 100-110 kDa (as indicated) in the parental HeLa cell line, but is not detectable in knockout HeLa cell line. GAPDH (Catalog # Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

## PREPARATION AND STORAGE

<b>Reconstitution</b>	Sterile PBS to a final concentration of 0.2 mg/mL.
<b>Shipping</b>	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
<b>Stability &amp; Storage</b>	<p><b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b></p> <ul style="list-style-type: none"> <li>• 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>• 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

## BACKGROUND

DNMT3B (DNA methyltransferase 3B; also DNA MTase HsaIIIB) 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.