Human DNMT1 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF6110

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human DNMT1 in Western blots.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human DNMT1 Ala1381-Ala1587 Accession # P26358
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.

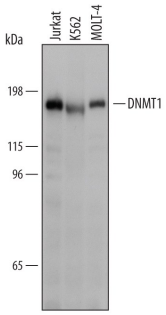
APPLICATIONS

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	1 µg/mL	See Below
Immunocytochemistry	1-15 µg/mL	See Below

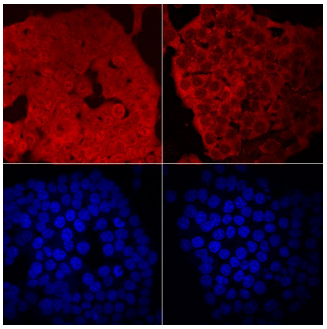
DATA

Western Blot



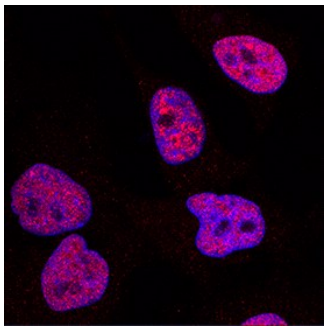
Detection of Human DNMT1 by Western Blot. Western blot shows lysates of Jurkat human acute T cell leukemia cell line, K562 human chronic myelogenous leukemia cell line, and MOLT-4 human acute lymphoblastic leukemia cell line. PVDF Membrane was probed with 1 µg/mL of Sheep Anti-Human DNMT1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6110) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for DNMT1 at approximately 183 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



DNMT1 in HCT-116 Human Cell Line. DNMT1 was detected in immersion fixed HCT-116 human colorectal carcinoma cell line untreated (left panels) and treated (right panels) with 1 µM 5-azacytidine for 24 hours using Sheep Anti-Human DNMT1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6110) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red, upper panels; Catalog # NL010) and counterstained with DAPI (blue, lower panels). Specific staining was localized to nuclei and cytoplasm. Nuclear staining was reduced following treatment with 5-azacytidine. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunocytochemistry



DNMT1 in HeLa Human Cell Line. DNMT1 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Sheep Anti-Human DNMT1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6110) at 1.7 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
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.** ● 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.

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BACKGROUND

DNMT1 (DNA methyltransferase 1; also MCMT and CXXC-type Zn finger protein 9) is a 190 kDa member of the C5-methyltransferase family of enzymes. It is ubiquitous in expression, and appears during the S-phase of the cell cycle. DNMT1 catalyzes the attachment of a methyl group to the C5 position of CpG cytosines using AdoMet/adenosylmethionine as a co factor. This acts to silence select genes during X chromosome inactivation and tumorigenesis. Human DNMT1 is 1616 amino acids (aa) in length. It contains an N-terminal regulatory, homodimerization and target recognition region (aa 1-1100), a Gly-Lys linker segment (aa 1109-1120) and a catalytic domain (aa 1121-1616). It is acetylated, phosphorylated, and SUMOylated. There are multiple splice variants. There is a deletion of aa 1-336, alternative start sites at Met122 and 62 aa upstream of the standard start site, and a 17 aa substitution for Pro149. Over aa 1381-1587, human DNMT1 shares 87% aa identity with mouse DNMT1.

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