Human Mcl-1 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF8281

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Mcl-1 in direct ELISAs and Western blots.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human Mcl-1 Val147-Gly219 Accession # Q07820
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 either lyophilized or as a 0.2 µm filtered solution in PBS.

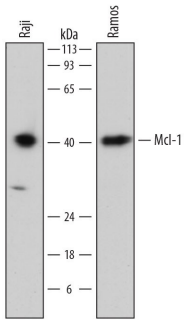
APPLICATIONS

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.*

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Simple Western	20 µg/mL	Raji human Burkitt's lymphoma cell line.
Knockout Validated	Mcl-1 is specifically detected in A431 human epithelial carcinoma parental cell line but is not detectable in Mcl-1 knockout A431 cell line.	

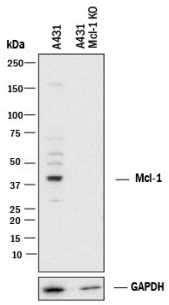
DATA

Western Blot



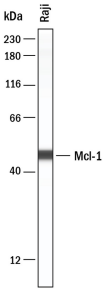
Detection of Human Mcl-1 by Western Blot. Western blot shows lysates of Raji human Burkitt's lymphoma cell line and Ramos human Burkitt's lymphoma cell line. PVDF membrane was probed with 0.5 µg/mL of Sheep Anti-Human Mcl-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8281) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for Mcl-1 at approximately 40 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Knockout Validated



Western Blot Shows Human Mcl-1 Specificity by Using Knockout Cell Line. Western blot shows lysates of A431 human epithelial carcinoma parental cell line and Mcl-1 knockout A431 cell line (KO). PVDF membrane was probed with 0.5 µg/mL of Sheep Anti-Human Mcl-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8281) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for Mcl-1 at approximately 40 kDa (as indicated) in the parental A431 cell line, but is not detectable in knockout A431 cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Simple Western



Detection of Human Mcl-1 by Simple Western™. Simple Western lane view shows lysates of Raji human Burkitt's lymphoma cell line, loaded at 0.2 mg/mL. A specific band was detected for Mcl-1 at approximately 48 kDa (as indicated) using 20 µg/mL of Sheep Anti-Human Mcl-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8281) . This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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Catalog Number: AF8281

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.** • 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

MCL-1 (induced Myeloid Cell Leukemia differentiation protein 1; also Bcl2L3 and mcl1/EAT) is a 40-45 kDa member of the Bcl-2 family of proteins. It is widely expressed (in B cells, T cells, neutrophils and fibroblasts) and classified as a prosurvival Bcl-2 family member. Functionally, full-length MCL-1 sequesters the proapoptotic proteins Bak and Bax, rendering them inactive. It also delays cell-cycle progression by interacting with CDK1, CHK1 and PCNA. Human MCL-1 is a likely a 350 amino acid (aa) type II transmembrane protein. It contains a large cytoplasmic region (aa 1-327) plus a very short 2 aa C-terminal luminal segment. The cytoplasmic region has multiple domains, including a PEST (Pro/Glu/Ser/Thr)-like segment (aa 104-175), four ubiquitination sites, at least six utilized phosphorylation sites, and three Bcl2-like homology domains (aa 209-223; 252-272; 304-319). MCL-1 is known to form homodimers. There is one splice variant. It is 32-33 kDa in size and contains a 42 aa substitution for aa 230-350. This short form heterodimerizes with full-length MCL-1, rendering it incapable of interacting with Bak and Bax. MCL-1 also undergoes caspase processing. Cleavage after Asp127 generates 17 and 28-30 kDa fragments, while cleavage after Asp 157 generates 21 and 23-25 kDa fragments. These fragments give rise to a proapoptotic environment. Over aa 147-219, human MCL-1 shares 74% aa sequence identity with mouse Mcl-1.

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