Human HOIP/RNF31 Antibody

Monoclonal Mouse IgG_1 Clone # 875227

Catalog Number: MAB8039

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human HOIP/RNF31 in direct ELISA and Western Blot.
Source	Monoclonal Mouse IgG_1 Clone # 875227
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived recombinant human HOIP/RNF31 Arg970-Lys1072 Accession # Q96EP0
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.

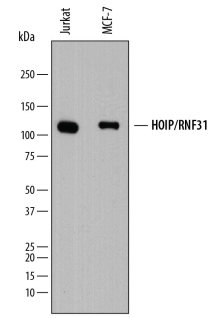
APPLICATIONS

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	0.1 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Simple Western	5 µg/mL	See Below

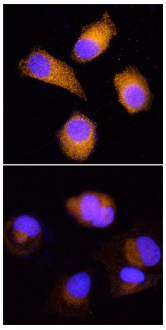
DATA

Western Blot



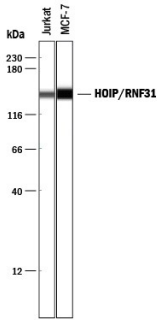
Detection of Human HOIP/RNF31 by Western Blot. Western blot shows lysates of Jurkat human acute T cell leukemia cell line and MCF-7 human breast cancer cell line. PVDF membrane was probed with 0.5 µg/mL of Mouse Anti-Human HOIP/RNF31 Monoclonal Antibody (Catalog # MAB8039) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for HOIP/RNF31 at approximately 120 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



HOIP/RNF31 in MCF-7 Human Cell Line. HOIP/RNF31 was detected in immersion fixed MCF-7 human breast cancer cell line, untreated (upper panel) or treated with Proteasome Inhibitor I (Tocris Bioscience, Catalog # 4045, lower panel), using Mouse Anti-Human HOIP/RNF31 Monoclonal Antibody (Catalog # MAB8039) at 25 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Simple Western



Detection of Human HOIP/RNF31 by Simple Western™. Simple Western lane view shows lysates of Jurkat human acute T cell leukemia cell line and MCF-7 human breast cancer cell line, loaded at 0.5 mg/mL. A specific band was detected for HOIP/RNF31 at approximately 144 kDa (as indicated) using 5 µg/mL of Mouse Anti-Human HOIP/RNF31 Monoclonal Antibody (Catalog # MAB8039). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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

RNF31 (RING [Really INteresting Gene] Finger Protein 31; also HOIL-1-interacting protein/HOIP, and Zn in-between-RING-finger ubiquitin-associated domain protein/ZIBRA) is a cytoplasmic E3 ubiquitin-protein ligase that is found in breast epithelium and multiple cancer types. Although its predicted MW is 102 kDa, it runs anomalously at 95-135 kDa in SDS-Page. Ubiquitin (Ub) chains are typically thought of as 9 kDa additions to Lys residues of target molecules. The activity associated with Ub addition depends upon the location of the attachment, and the monomeric vs. polymeric nature of the chains. Ub can also be added to N-terminal Met residues by an intracellular complex called LUBAC (Linear Ub chain Assembly Complex). This complex is key to NFκB pathway activation. Following exposure of cells to cytokines, LUBAC ubiquitinates NEMO, which subsequently induces IKKβ phosphorylation, IκBα degradation, and NFκB translocation into the nucleus with gene activation. The LUBAC complex contains RNF31, HOIL-1L and sharpin, and it is now known that RNF31 is the catalyst for linear Ub chain formation. Human RNF31 is 1072 amino acids (aa) in length. It contains three consecutive RanBP2-type Zn finger domains (aa 299-438), a utilized phosphoryation site at Ser466, one UBA domain (aa 564-615), and two RING-type Zn finger domains (aa 699-747 and 860-909) with an intervening IBR-type Zn finger region (aa 779-841). There are at least two isoform variants, one that contains a 13 aa substitution for aa 1-164, and another that shows a deletion of aa 73-630 coupled to a deletion of aa 833-841. Human RNF31 shares 87% aa sequence identity with mouse RNF31.

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