Human/Mouse/Rat p62/SQSTM1 Antibody

Monoclonal Mouse IgG_1 Clone # 864807

Catalog Number: MAB8028

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human p62/SQSTM1 in ELISAs. Detects human, mouse and rat p62/SQSTM1 in Western blots
Source	Monoclonal Mouse IgG_1 Clone # 864807
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived recombinant human p62/SQSTM1 Asp368-Leu440 Accession # Q13501
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.

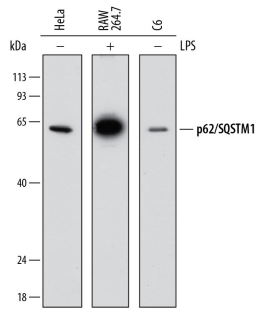
APPLICATIONS

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	2 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Immunohistochemistry	5-25 µg/mL	Immersion fixed paraffin-embedded sections of human liver
Immunoprecipitation	1 µg/1 mg cell lysate	Cell lysate of U2OS human osteosarcoma cell line
Simple Western	20 µg/mL	See Below
Knockout Validated	p62/SQSTM1 is specifically detected in HeLa human cervical epithelial carcinoma parental cell line and parental U2OS cell line but is not detectable in p62/SQSTM1 knockout HeLa cell line and knockout U2OS cell line.	

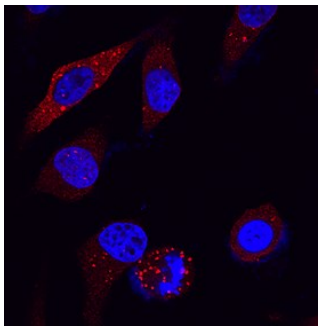
DATA

Western Blot



Detection of Human, Mouse, and Rat p62/SQSTM1 by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line, RAW 264.7 mouse monocyte/macrophage cell line, and C6 rat glioma cell line untreated (-) or treated (+) with 1 µg/mL LPS for 24 hours. PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human/Mouse/Rat p62/SQSTM1 Monoclonal Antibody (Catalog # MAB8028) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for p62/SQSTM1 at approximately 62 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



p62/SQSTM1 in HeLa Human Cell Line. p62/SQSTM1 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Mouse Anti-Human/Mouse/Rat p62/SQSTM1 Monoclonal Antibody (Catalog # MAB8028) 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 phagosomes in cell cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

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Monoclonal Mouse IgG_1 Clone # 864807

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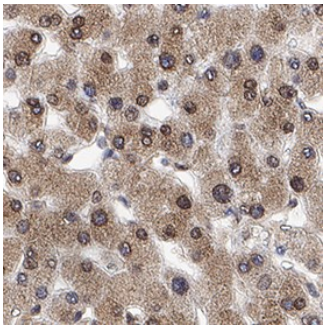
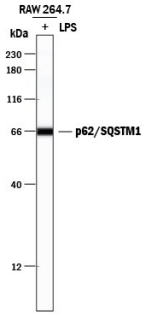
Immunohistochemistry



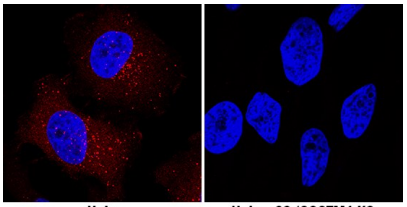
p62/SQSTM1 in Human Liver. p62/SQSTM1 was detected in immersion fixed paraffin-embedded sections of human liver using Mouse Anti-Human/Mouse/Rat p62/SQSTM1 Monoclonal Antibody (Catalog # MAB8028) at 5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC001). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to nuclei and cytoplasm in hepatocytes. Staining was performed using our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Simple Western



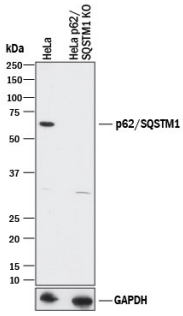
Detection of Mouse p62/SQSTM1 by Simple Western™. Simple Western lane view shows lysates of RAW 264.7 mouse monocyte/macrophage cell line untreated (-) or treated (+) with 1 µg/mL LPS for 24 hours, loaded at 0.2 mg/mL. A specific band was detected for p62/SQSTM1 at approximately 66 kDa (as indicated) using 20 µg/mL of Mouse Anti-Human/Mouse/Rat p62/SQSTM1 Monoclonal Antibody (Catalog # MAB8028). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

Knockout Validated



p62/SQSTM1 Specificity is Shown by Immunocytochemistry in Knockout Cell Line. p62/SQSTM1 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line but is not detected in p62/SQSTM1 knockout (KO) HeLa cell line using Mouse Anti-Human/Mouse/Rat p62/SQSTM1 Monoclonal Antibody (Catalog # MAB8028) at 3 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.

Knockout Validated



Western Blot Shows Human p62/SQSTM1 Specificity Using Knockout Cell Line. Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and p62/SQSTM1 knockout HeLa cell line (KO). PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human/Mouse/Rat p62/SQSTM1 Monoclonal Antibody (Catalog # MAB8028) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for p62/SQSTM1 at approximately 62 kDa (as indicated) in the parental HeLa cell line, but is not detectable in the knockout HeLa cell line. GAPDH (Catalog # MAB5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

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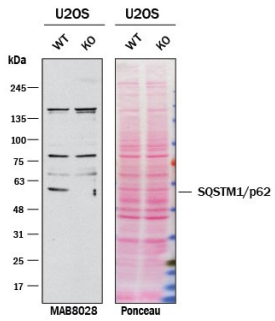
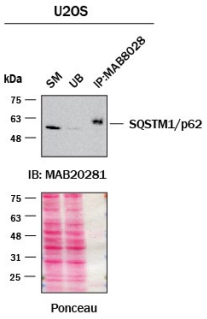


Western Blot Shows Human p62/SQSTM1 Specificity Using Knockout Cell Line. Western blot shows lysates of U2OS human osteosarcoma cell line and p62/SQSTM1 knockout U2OS cell line (KO). Nitrocellulose membrane was probed with 0.5 µg/mL of Mouse Anti-Human/Mouse/Rat p62/SQSTM1 Monoclonal Antibody (Catalog # MAB8028) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody. A specific band was detected for p62/SQSTM1 at approximately 62 kDa (as indicated) in the parental U2OS cell line, but is not detectable in knockout U2OS cell line. The Ponceau stained transfer of the blot is shown. This experiment was conducted under reducing conditions. Image, protocol, and testing courtesy of YCharOS Inc. See ycharos.com for additional details.

Immunoprecipitation



Detection of SQSTM1/p62 by Immunoprecipitation Immunoprecipitation was performed on cell lysate of U2OS human osteosarcoma cell line using 1.0 µg of Mouse Anti-Human SQSTM1/p62 Monoclonal Antibody (Catalog # MAB8028) pre-coupled to protein G or protein A beads. Immunoprecipitated SQSTM1/p62 was detected with Rabbit Anti-SQSTM1/p62 Monoclonal Antibody (Catalog # MAB80281). The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitated. Image, protocol, and testing courtesy of YCharOS Inc. (ycharos.com).

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.5 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

SQSTM1 (Sequestrome-1), also called p62, is a widely expressed, stress-inducible, multifunctional 62 kDa intracellular protein. The 440 amino acid (aa) human SQSTM1 contains multiple adaptor domains that allow interaction with proteins in NGF/NFkB and other signaling pathways (notably TRAF6, atypical protein kinase C family and Src family), polyubiquitin, proteasome subunits and many others. It contains numerous regulatory phosphorylation sites and a dimerization site. SQSTM1 shuttles ubiquitinylated proteins to the proteasome and is important in autophagy and apoptosis. Its dysregulation is associated with Paget's disease of bone, Parkinson's and Alzheimer's diseases, and cancers. Within aa 344-440, which includes the ubiquitin-binding domain, human SQSTM1 shares 100% aa sequence identity with mouse and rat SQSTM1.

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