Human/Mouse/Rat p62/SQSTM1 Antibody

Recombinant Monoclonal Mouse IgG_{2B} Clone # 864807R

Catalog Number: MAB8028R

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human p62/SQSTM1 in ELISAs. Detects human, mouse and rat p62/SQSTM1 in Western blots
Source	Recombinant Monoclonal Mouse IgG_{2B} Clone # 864807R
Purification	Protein A or G purified from cell 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.

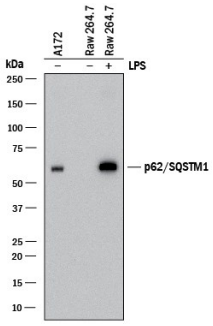
APPLICATIONS

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	1 µg/mL	See Below
Immunocytochemistry	3-25 µg/mL	See Below
Immunoprecipitation	1 µg/1 mg cell lysate	Cell lysate of U2OS human osteosarcoma cell line
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.	

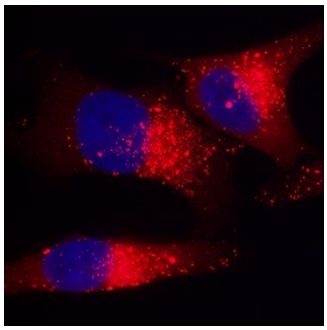
DATA

Western Blot



Detection of Human and Mouse p62/SQSTM1 by Western Blot. Western blot shows lysates of A172 human glioblastoma cell line and RAW 264.7 mouse monocyte/macrophage cell line untreated (-) or treated (+) with 10 µg/mL LPS for 4 hours. PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human/Mouse/Rat p62/SQSTM1 Monoclonal Antibody (Catalog # MAB8028R) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # 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 treated with staurosporine using Mouse Anti-Human/Mouse/Rat p62/SQSTM1 Monoclonal Antibody (Catalog # MAB8028R) 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 # Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

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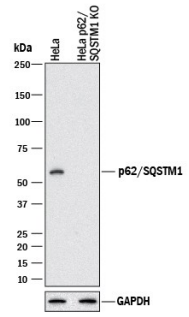
Human/Mouse/Rat p62/SQSTM1 Antibody

Recombinant Monoclonal Mouse IgG_{2B} Clone # 864807R

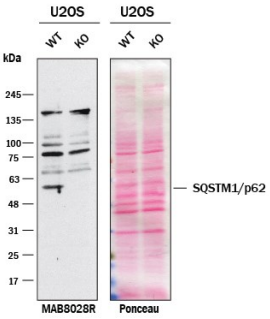
Catalog Number: MAB8028R

Knockout Validated



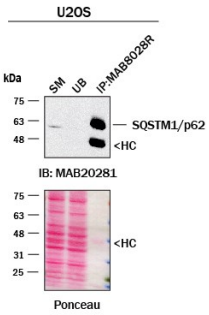
Western Blot Shows Human p62/SQSTM1 Specificity Using Knockout Cell Line. Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and p62/SQSTM1 knockout HeLa cell line (KO). PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human/Mouse/Rat p62/SQSTM1 Monoclonal Antibody (Catalog # MAB8028) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # 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 # Catalog # MAB5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Knockout Validated



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 # MAB8028R) 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 # MAB8028R) 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	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.

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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