

Purified anti-p62 (SQSTM1) Antibody (Previously Covance catalog# MMS-5034)

Catalog# / Size 814802 / 25 μL

814801 / 100 µL

Clone 1B5.H9

Regulatory Status RUO

Other Names P60, p62, A170, OSIL, PDB3, ZIP3, p62B, sequestosome-1, oxidative stress-induced-like

ubiquitin-binding protein p62

Previously Covance Catalog# MMS-5034

Isotype Mouse IgG1, κ

Description The p62 protein, also called sequestosome 1 (SQSTM1), is a ubiquitin-binding scaffold protein

that colocalizes with ubiquitinated protein aggregates in many neurodegenerative diseases and proteinopathies of the liver. The protein is able to polymerize via an N-terminal PB1 domain and can interact with ubiquitinated proteins via the C-terminal UBA domain. Also, p62/SQSTM1 binds directly to LC3 and GABARAP family proteins via a specific sequence motif. The protein is itself degraded by autophagy and may serve to link ubiquitinated proteins to the autophagic machinery to enable their degradation in the lysosome. Recent studies have revealed a novel

function for p62 in innate immunity.

Product Details

Verified Reactivity Human, Mouse, Rat

Antibody Type Monoclonal
Host Species Mouse

Immunogen This monoclonal antibody was raised against a peptide sequence corresponding to amino acids

201-212 of the human SQSTM 1 (isoform 1) protein conjugated to KLH.

Formulation Phosphate-buffered solution.

Preparation The antibody was purified by affinity chromatography.

Concentration 1 mg/mL

Storage & Handling The antibody solution should be stored undiluted between 2°C and 8°C. Please note the storage

condition for this antibody has been changed from -20°C to between 2°C and 8°C. You can also check your vial or your CoA to find the most accurate storage condition for this antibody.

Application <u>IHC-P - Quality tested</u>

WB, ICC - Verified

Recommended Usage Each lot of this antibody is quality control tested by formalin-fixed paraffin-embedded

immunohistochemical staining. For immunohistochemistry, a concentration range of 10 - 15 $\mu g/mL$ is suggested. For Western blotting, the suggested use of this reagent is 1.0 - 10 μg per mL. For immunocytochemistry, a concentration range of 1.0 - 10 $\mu g/mL$ is recommended. It is recommended that the reagent be titrated for optimal performance for each application.

Application Notes This antibody is effective in immunohistochemistry on formalin-fixed paraffin-embedded tissue (IHC-

P), immunoblotting (WB), and immunocytochemistry (ICC).

Product Citations

1. Zhou B, et al. 2020. Nat Microbiol. 5:1576. PubMed

2. Tapia D, et al. 2019. Nat Commun. 10:735. PubMed

3. Trejo-Lopez JA, et al. 2020. J Neuropathol Exp Neurol. 407:79. PubMed

4. Quach C, et al. 2019. Nat Commun. 4.361805556. PubMed

RRID AB 2728534 (BioLegend Cat. No. 814802)

AB_2564787 (BioLegend Cat. No. 814801)

Antigen Details

Structure p62 (SQSTM1) is a 440 amino acid protein with an expected molecular mass of 62 kD.

Biology Area Cell Biology, Neurodegeneration, Neuroscience, Neuroscience Cell Markers, Protein Trafficking

and Clearance

Molecular Family Adaptor Proteins, Autophagosome Markers

Gene ID <u>8878</u>

Related Protocols

Immunocytochemistry Staining Protocol

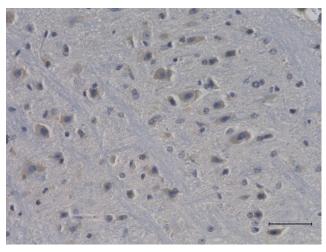
Western Blotting Protocol

Immunohistochemistry Protocol for Paraffin-Embedded Sections

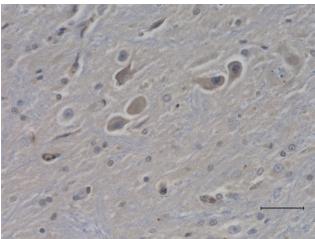
Other Formats

Purified anti-p62 (SQSTM1)

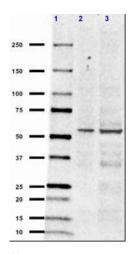
Product Data

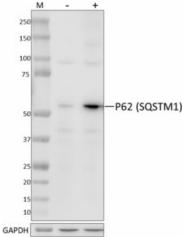


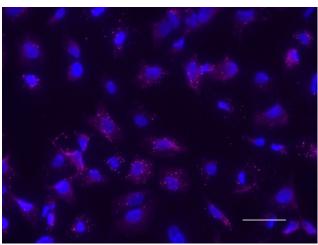
IHC staining of purified anti-p62 (SQSTM1) antibody (clone 1B5.H9) on formalin-fixed paraffin-embedded mouse brain tissue. Following antigen retrieval using Sodium Citrate H.I.E.R., the tissue was incubated with 15 μ g/ml of the primary antibody overnight at 4°C. BioLegend's Ultra-Streptavidin (USA) HRP kit (Multi-Species, DAB, Cat. No. 929901) was used for detection followed by hematoxylin counterstaining, according to the protocol provided. The image was captured with a 40X objective. Scale bar: 50 μ m

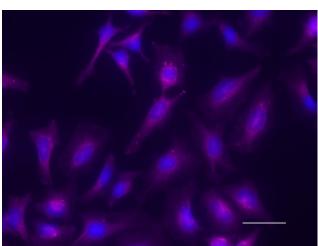


IHC staining of purified anti-p62 (SQSTM1) antibody (clone 1B5.H9) on formalin-fixed paraffin-embedded rat brain tissue. Following antigen retrieval using Sodium Citrate H.I.E.R., the tissue was incubated with 15 μ g/ml of the primary antibody overnight at 4°C. BioLegend's Ultra-Streptavidin (USA) HRP kit (Multi-Species, DAB, Cat. No. 929901) was used for detection followed by hematoxylin counterstaining, according to the protocol provided. The image was captured with a 40X objective. Scale bar: 50 μ m









Western blot of purified anti-p62 (SQSTM1) antibody (clone 1B5.H9). Lane 1: Molecular weight marker; Lane 2: 20 µg of HEK293 cell lysate; Lane 3: 20 µg of SH-SY5Y cell lysate. The blot was incubated with 5 µg/mL of the primary antibody overnight at 4°C, followed by incubation with HRP labeled goat anti-mouse IgG (Cat. No. 405306). Enhanced chemiluminescence was used as the detection system.

Whole cell extracts (15 µg protein) from HeLa cells untreated (-) or treated (+) with 50 µM chloroquine phosphate (Cat. No. 427502) for 18 hours were resolved by 4-12% Bis-Tris gel electrophoresis, transferred to a PVDF membrane, and probed with 1.0 µg/mL purified anti-p62 (SQSTM1) antibody (clone 1B5.H9) overnight at 4°C. Proteins were visualized by chemiluminescence detection using HRP donkey anti-mouse IgG antibody (Cat. No. 405306) at a 1:3000 dilution. Western-Ready™ ECL Substrate Premium Kit (Cat. No. 426319) was used as a detection agent. Direct-Blot™ HRP anti-GAPDH antibody (Cat. No. 607904) was used as a loading control at a 1:25000 dilution (lower). Lane M: Molecular weight marker.

ICC staining of purified anti-p62 (SQSTM1) antibody (clone 1B5.H9) on A549 cells. The cells were fixed with 4% PFA, permeabilized with a buffer containing 0.1% Triton X-100 and 0.25% BSA, and blocked with 2% normal goat serum and 0.02% BSA. The cells were then incubated with 10 µg/ml of the primary antibody for overnight at 4°C, followed by incubation with 5 µg/ml of Alexa Fluor® 594 goat anti-Mouse IqG for one hour at room temperature. Nuclei were counterstained with DAPI, and the slides were mounted with ProLong™ Gold Antifade Mountant. The image was captured with a 40X objective. Scale bar: 50 µm

ICC staining of purified anti-p62 (SQSTM1) antibody (clone 1B5.H9) on HeLa cells. The cells were fixed with 4% PFA, permeabilized with a buffer containing 0.1% Triton X-100 and 0.25% BSA, and blocked with 2% normal goat serum and 0.02% BSA. The cells were then incubated with 10 µg/ml of the primary antibody for overnight at 4°C, followed by incubation with 5 µg/ml of Alexa Fluor® 594 goat anti-Mouse IgG for one hour at room temperature. Nuclei were counterstained with DAPI, and the slides were mounted with ProLong™ Gold Antifade Mountant. The image was captured with a 40X objective. Scale bar: 50 µm

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BioLegend Inc., 8999 BioLegend Way, San Diego, CA 92121 www.biolegend.com Toll-Free Phone: 1-877-Bio-Legend (246-5343) Phone: (858) 768-5800 Fax: (877) 455-9587