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Datasheet for 600-401-EU6

SQSTM1 Antibody

Overview

Description:	Anti-SQSTM1 (RABBIT) Antibody - 600-401-EU6
Item No.:	600-401-EU6
Size:	100 µg
Applications:	ELISA, IF, IHC, WB
Reactivity:	Human, Mouse, Rat
Host Species:	Rabbit

Product Details

Background:	SQSTM1/p62 is an adapter protein which binds ubiquitin and regulates signaling cascades through ubiquitination. It may regulate the activation of NF-kB by TNF-a, nerve growth factor (NGF) and interleukin-1. SQSTM1/p62, a co-interacting protein of the atypical PKC isoforms, has a UBA domain at its C-terminal end, which binds non-covalently to polyubiquitin chain. SQSTM1's UBA domain is necessary for recruitment of polyubiquitin and aggresome formation. SQSTM1 may play a role in titin/TTN downstream signaling in muscle cells and may be involved in cell differentiation, apoptosis, immune response and regulation of K+ channels. Mutations in the ubiquitin-associated (UBA) domain of SQSTM1 commonly cause Paget's disease of bone since the UBA is necessary for aggregate sequestration and cell survival.
Synonyms:	SQSTM1 Antibody, p60, p62, A170, OSIL, PDB3, ZIP3, p62B, ORCA, Sequestosome-1, EBI3-associated protein of 60 kDa, EBIAP
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	SQSTM1
Reactivity:	Human, Mouse, Rat
Immunogen Type:	Conjugated Peptide

Immunogen:	Anti-SQSTM1 antibody was prepared from whole rabbit serum produced by repeated immunizations with a 13 amino acid synthetic peptide from near the C-terminus of human SQSTM1. The immunogen is located within the last 50 amino acids of SQSTM1.
Purity/Specificity:	Anti-SQSTM1 Antibody was affinity purified from monospecific antiserum by immunoaffinity chromatography. Cross reactivity with SQSTM1 from other sources has not been determined. Human SQSTM1 has two isoforms, Mouse SQSTM1 has two isoforms, Rat SQSTM1 has three isoforms. Anti-SQSTM1 Antibody can detect the isoforms of human, mouse, and rat except the shortest rat isoform 3.
Relevant Links:	• UniProtKB - Q13501 • GeneID - 8878 • NCBI - NP_003891.1

Application Details

Tested Applications:	ELISA, IF, IHC, WB
Application Note:	Anti-SQSTM1 Antibody has been tested for use in ELISA, Western Blotting, Immunohistochemistry-P, and Immunofluorescence. Expect a band at approximately 48 kDa, observed ~65kDa in western blots. Specific conditions for reactivity should be optimized by the end user. Positive control: Human spleen tissue, rat spleen tissue, rat liver tissue, mouse skeletal muscle tissue, mouse pancreas tissue.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 1:20,000
IF:	20 µg/mL
IHC:	2-5 µg/mL
WB:	0.5-2 µg/mL

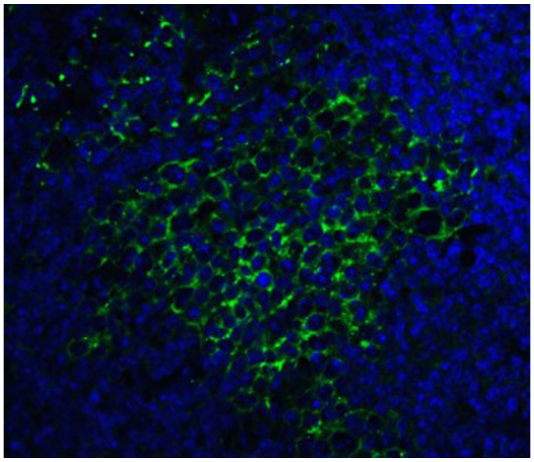
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.01 M Sodium Phosphate, 0.25 M Sodium Chloride, pH 7.2
Preservative:	0.02% (w/v) Sodium Azide
Stabilizer:	None

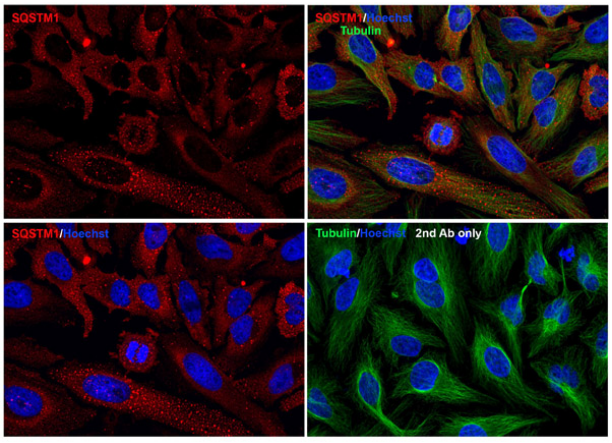
Shipping & Handling

Shipping Condition:	Wet Ice
Storage Condition:	Antibody can be stored at 4°C up to one year. Antibodies should not be exposed to prolonged high temperatures.
Expiration:	Expiration date is one (1) year from date of receipt.

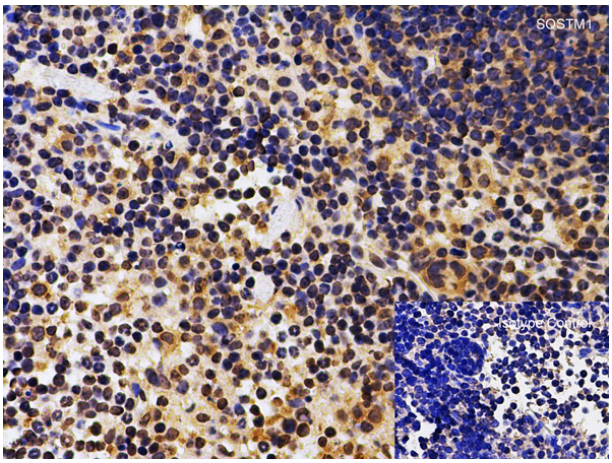
Images



Immunofluorescence Microscopy
Immunofluorescence Validation of SQSTM1.
Tissue: Mouse Spleen Tissue.
Fixation: 4% paraformaldehyde-fixed.
Labeling: SQSTM1 at 20 µg/mL, followed by goat anti-rabbit IgG secondary antibody at 1:500 dilution (green) and DAPI staining (blue).

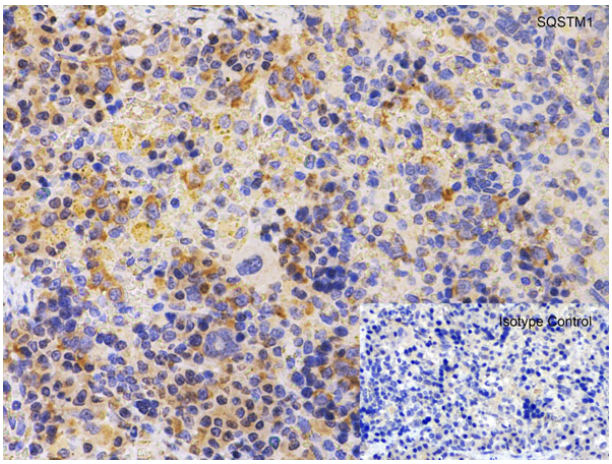


Immunofluorescence Microscopy
Immunofluorescence Validation of SQSTM1.
Cell: HeLa Cells.
Fixation: PFA-fixed.
Labeling: SQSTM1 at 20 µg/mL, followed by goat anti-rabbit IgG secondary antibody at 1:1000 dilution (red) and Hoechst staining (blue). Alpha tubulin was stained with anti-alpha tubulin antibody following by goat anti-mouse IgG secondary antibody (green). Images were captured with confocal microscopy.

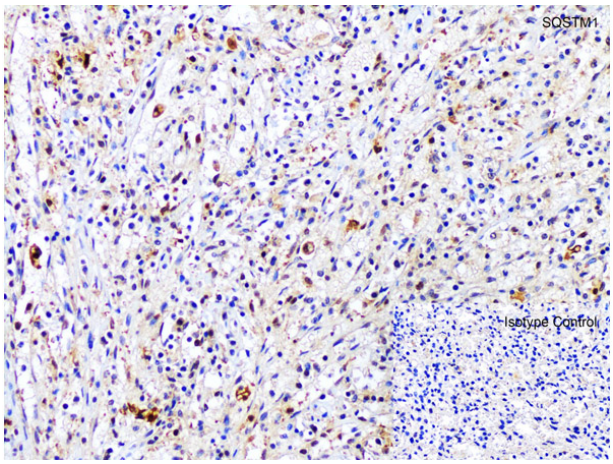


Immunohistochemistry
Immunohistochemistry Validation of SQSTM1.
Tissue: Mouse Spleen Tissue.
Fixation: paraffin-embedded, formaldehyde and blocked with 10% serum for 1 h at RT.
Antigen retrieval: heat mediation with a citrate buffer (pH6).

Primary Antibody: anti-SQSTM1 antibody at 2 µg/ml overnight at 4°C. Secondary: goat anti-rabbit IgG H&L (HRP) at 1:250. Counter stained with Hematoxylin.



Immunohistochemistry
Immunohistochemistry Validation of SQSTM1.
Cell: Rat Spleen Cells.
Fixation: 4% paraformaldehyde-fixed.
Labeling: SQSTM1 at 20 µg/mL, followed by goat anti-rabbit IgG secondary antibody at 1:500 dilution (red).

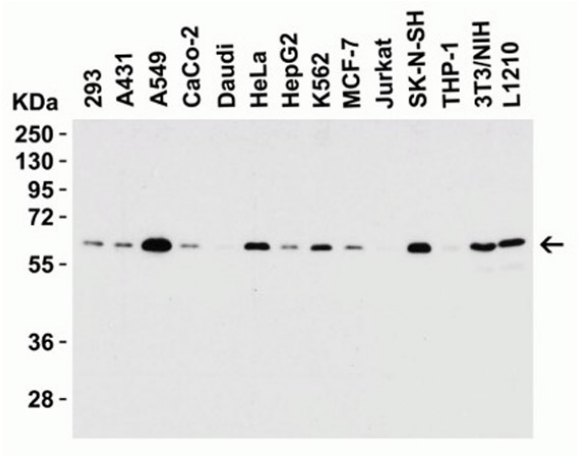


Immunohistochemistry
Immunohistochemistry Validation of SQSTM1.
Tissue: Human Spleen Tissue.
Fixation: paraffin-embedded, formaldehyde and blocked with 10% serum for 1 h at RT.
Antigen retrieval: heat mediation with a citrate buffer (pH6).

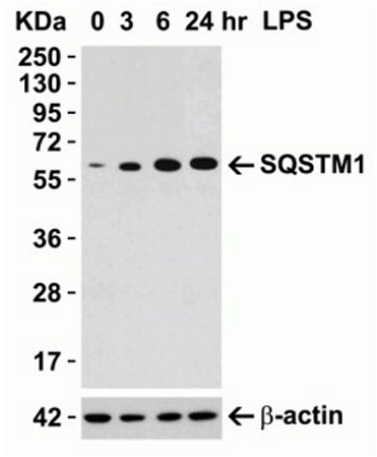
Primary Antibody: anti-SQSTM1 antibody at 2 µg/ml overnight at 4°C. Secondary: goat anti-rabbit IgG H&L (HRP) at 1:250. Counter stained with Hematoxylin.



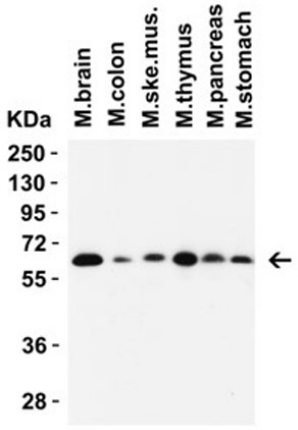
Western Blot
KO Validation of SQSTM1.
Load: 10 µg of HEK293T WT cell lysates or SQSTM1 KO cell lysates.
Primary antibody: SQSTM1 at 1µg/mL and beta-actin at 1µg/mL for 1 hr incubation at RT in 5% NFDM/TBST.
Secondary: Goat Anti-Rabbit IgG HRP conjugate at 1:10000 dilution.



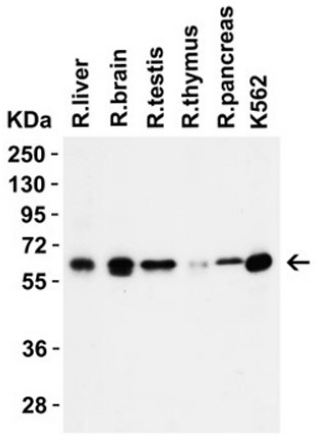
Western Blot
Western Blot Validation of SQSTM1.
Load: 15 µg of lysates per lane.
Lane 1: 293, lane 2: A431, lane 3: A549, lane 4: Caco-2, lane 5: Daudi, lane 6: HeLa, lane 7: HepG2, lane 8: K562, lane 9: MCF-7, lane 10: Jurkat, lane 11: SK-N-SH, lane 12: THP-1, lane 13: 3T3/NIH, lane 14: L1210.
Primary antibody: SQSTM1 at 0.5µg/mL for 1h incubation at RT in 5% NFDM/TBST.
Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.



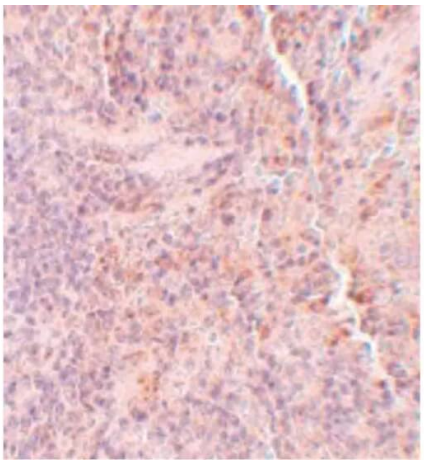
Western Blot
Western Blot Validation of SQSTM1.
Load: 15 µg of Raw 264.7 Mouse Macrophage cell lysates per lane. Raw 264.7 cells were treated with LPS (0.3 µg /mL) for different time period (0-24 hrs).
Primary antibody: SQSTM1 at 0.5 µg/mL for 1h incubation at RT in 5% NFDM/TBST.
Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.
There was an increase in SQSTM1 protein expression overtime after LPS treatment.



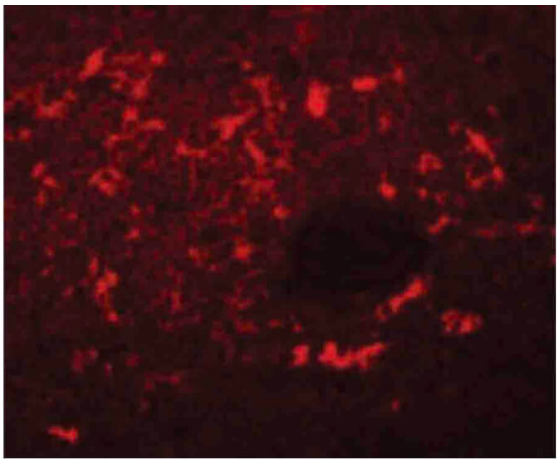
Western Blot
Western Blot Validation of SQSTM1.
Load: 15 µg of mouse tissue lysates per lane.
Lane 1: mouse brain, Lane 2: mouse colon, Lane 3: mouse skeletal muscle, Lane 4: mouse thymus, Lane 5: mouse panceas, Lane 6: mouse stomach.
Primary antibody: SQSTM1 at 1µg/mL for 1h incubation at RT in 5% NFDM/TBST.
Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.



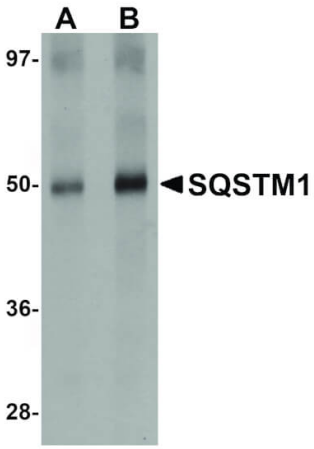
Western Blot
Western Blot Validation of SQSTM1.
Load: 15 µg of rat tissue lysates per lane.
Lane 1: rat liver, Lane 2: rat brain, Lane 3: rat testis, Lane 4: rat thymus, Lane 5: rat pancreas, Lane 6: K562.
Primary antibody: SQSTM1 at 1µg/mL for 1h incubation at RT in 5% NFDM/TBST.
Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.



Immunohistochemistry
Immunohistochemistry of SQSTM1 antibody. Tissue: rat spleen tissue. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: SQSTM1 antibody at 5 µg/mL for 1 h at RT. Secondary antibody: Peroxidase rabbit secondary antibody at 1:10,000 for 45 min at RT. Localization: SQSTM1 is localized in the intracellular space. Staining: SQSTM1 as precipitated pink signal with purple nuclear counterstain.



Immunofluorescence Microscopy
Immunofluorescence Microscopy of SQSTM1 antibody. Tissue: Rat spleen cells. Fixation: 0.5% PFA. Antigen retrieval: not required. Primary antibody: SQSTM1 antibody at 20 µg/mL for 1 h at RT. Secondary antibody: Fluorescein rabbit secondary antibody at 1:10,000 for 45 min at RT. Localization: SQSTM1 is localized in the intracellular space. Staining: SQSTM1 as red fluorescent signal.



Western Blot
Western Blot of SQSTM1 antibody. Lane 1: Human spleen tissue lysate with SQSTM1 antibody at 1 µg/mL. Lane 2: Human spleen tissue lysate with SQSTM1 antibody at 2 µg/mL. Secondary antibody: Peroxidase rabbit secondary antibody at 1:10,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C. Predicted/Observed size: 48 kDa, 50 kDa for SQSTM1. Other band(s): SQSTM1 splice variants and isoforms.

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