

## Anti-SQSTM1/p62 Antibody Picoband® (monoclonal, 3H11)

Catalog Number: M00300-1

### About SQSTM1

SQSTM1 (Sequestosome-1), also known as Ubiquitin-Binding Protein P62 or P62, is a protein that in humans is encoded by the SQSTM1 gene. The Src homology type 2 (SH2) domain is a highly conserved motif of about 100 amino acids which mediates protein-protein interactions by binding to phosphotyrosine. p56-lck, a T-cell-specific src family tyrosine kinase with an SH2 domain, is involved in T-cell signal transduction. The International Radiation Hybrid Mapping Consortium mapped the p62 gene to chromosome 5q35. Park et al. (1995) found that the p56-lck SH2 domain binds to p62 at the ser59 of p62 only when that serine is phosphorylated. Joung et al. (1996) expressed epitope-tagged p62 in HeLa cells and showed that the expressed protein bound to the lck SH2 domain and that this binding was dependent on the N-terminal 50 amino acids of p62 but not on the tyrosine residue in this region.

### Overview

Product Name	Anti-SQSTM1/p62 Antibody Picoband® (monoclonal, 3H11)
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-SQSTM1/p62 Antibody Picoband® (monoclonal, 3H11) catalog # M00300-1. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Monoclonal 3H11
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na <sub>2</sub> HPO <sub>4</sub> , 0.05mg NaN <sub>3</sub> .
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Mouse
Uniprot ID	Q13501

### Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human SQSTM1/p62, identical to the related mouse and rat sequences.
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Mouse IgG (EK1001) for Western blot, and HRP Conjugated anti-Mouse IgG Super Vision Assay Kit (SV0001-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Mouse IgG2a

Form	Lyophilized
Concentration	0
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.1-0.5ug/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, Mouse, Rat, By Heat Immunocytochemistry/Immunofluorescence, 2ug/ml, Human Flow Cytometry (Fixed), 1-3ug/1x10 <sup>6</sup> cells, Human

## Anti-SQSTM1/p62 Antibody Picoband® (monoclonal, 3H11) (M00300-1) Images

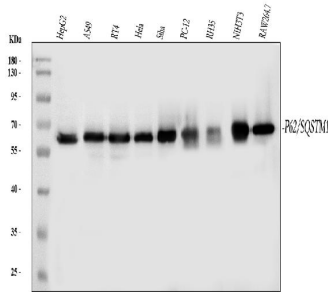


Figure 1. Western blot analysis of SQSTM1 using anti-SQSTM1 antibody (M00300-1).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: human HepG2 whole cell lysates,

Lane 2: human A549 whole cell lysates,

Lane 3: human RT4 whole cell lysates,

Lane 4: human Hela whole cell lysates,

Lane 5: human SiHa whole cell lysates,

Lane 6: rat PC-12 whole cell lysates,

Lane 7: rat RH35 whole cell lysates,

Lane 8: mouse NIH/3T3 whole cell lysates,

Lane 9: mouse RAW264.7 whole cell lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes.

Blocked the membrane with 5% non-fat milk/TBS for 1.5

hour at RT. The membrane was incubated with mouse anti-SQSTM1

antigen affinity purified monoclonal antibody

(Catalog # M00300-1) at 0.5 ug/mL overnight at 4°C, then

washed with TBS-0.1%Tween 3 times with 5 minutes each

and probed with a goat anti-mouse IgG-HRP secondary

antibody at a dilution of 1:10000 for 1.5 hour at RT. The

signal is developed using an Enhanced Chemiluminescent

detection (ECL) kit (Catalog # EK1001) with Tanon 5200

system. A specific band was detected for SQSTM1 at

approximately 62 kDa. The expected band size for SQSTM1

is at 48 kDa.

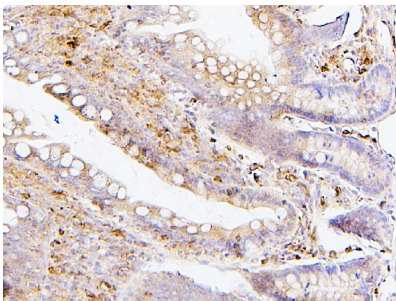


Figure 2. IHC analysis of SQSTM1 using anti-SQSTM1 antibody (M00300-1).

SQSTM1 was detected in section of rat intestine tissues.

Heat mediated antigen retrieval was performed in citrate

buffer (pH6, epitope retrieval solution) for 20 mins. The

tissue section was blocked with 10% goat serum. The tissue

section was then incubated with ug/ml mouse anti-SQSTM1

Antibody (M00300-1) overnight at 4°C. Biotinylated goat anti-

mouse IgG was used as secondary antibody and incubated for

30 minutes at 37°C. The tissue section was developed

using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021)

with DAB as the chromogen.

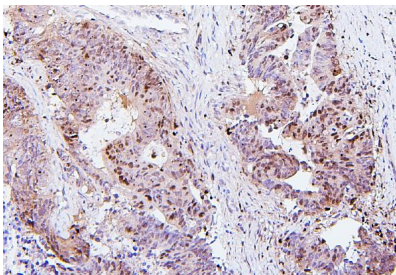


Figure 3. IHC analysis of SQSTM1 using anti-SQSTM1 antibody (M00300-1).

SQSTM1 was detected in section of human colon cancer

tissues. Heat mediated antigen retrieval was performed in

citrate buffer (pH6, epitope retrieval solution) for 20 mins.

The tissue section was blocked with 10% goat serum. The

tissue section was then incubated with ug/ml mouse anti-

SQSTM1 Antibody (M00300-1) overnight at 4°C. Biotinylated

goat anti-mouse IgG was used as secondary antibody and

incubated for 30 minutes at 37°C. The tissue section was

developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

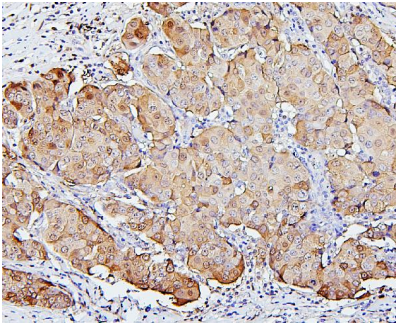


Figure 4. IHC analysis of SQSTM1 using anti-SQSTM1 antibody (M00300-1). SQSTM1 was detected in section of human mammary cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with ug/ml mouse anti-SQSTM1 Antibody (M00300-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

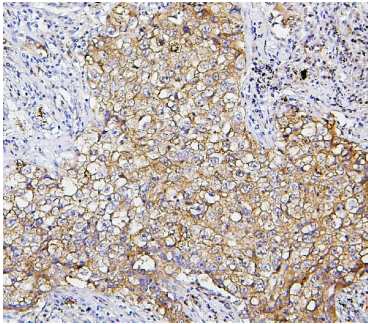


Figure 5. IHC analysis of SQSTM1 using anti-SQSTM1 antibody (M00300-1). SQSTM1 was detected in section of human lung cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with ug/ml mouse anti-SQSTM1 Antibody (M00300-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

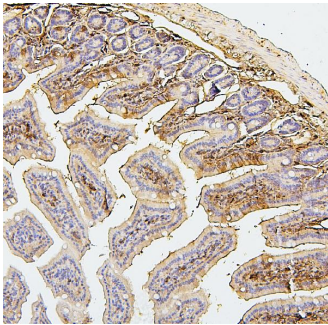


Figure 6. IHC analysis of SQSTM1 using anti-SQSTM1 antibody (M00300-1). SQSTM1 was detected in section of mouse intestine tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with ug/ml mouse anti-SQSTM1 Antibody (M00300-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

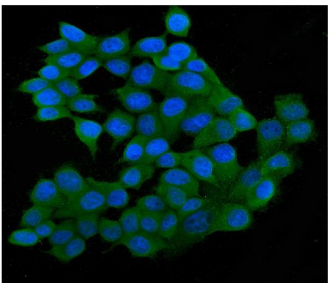


Figure 7. IF analysis of SQSTM1 using anti- SQSTM1 antibody (M00300-1). SQSTM1 was detected in immunocytochemical section of MCF7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2ug/mL mouse anti- SQSTM1 Antibody (M00300-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



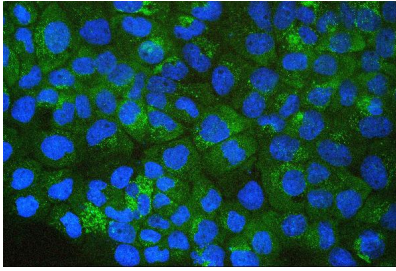


Figure 8. IF analysis of SQSTM1 using anti-SQSTM1 antibody (M00300-1).

SQSTM1 was detected in immunocytochemical section of A431 cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2ug/mL mouse anti-SQSTM1 Antibody (M00300-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

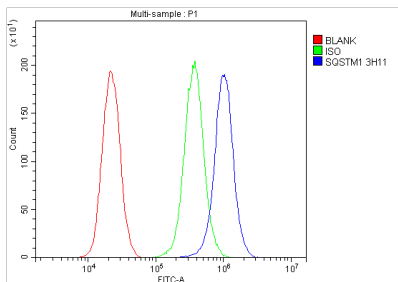


Figure 9. Flow Cytometry analysis of PC-3 cells using anti-SQSTM1 antibody (M00300-1).

Overlay histogram showing PC-3 cells stained with M00300-1 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-SQSTM1 Antibody (M00300-1, 1ug/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1ug/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

## 9 Publications Citing This Product

1. PubMed ID: 10.1093/jnen/nlaa007, Generation and Characterization of Novel Monoclonal Antibodies Targeting p62/sequestosome-1 Across Human Neurodegenerative Diseases
2. PubMed ID: -, Zhiwei Liao, Suyun Li, Rong Liu, Xiaobo Feng, Yunsong Shi, Kun Wang, Shuai Li, Yukun Zhang, Xinghuo Wu, Cao Yang, "Autophagic Degradation of Gasdermin D Protects against Nucleus Pulposus Cell Pyroptosis and Retards Intervertebral Disc Degeneration In Vivo", Oxidative Medicine and Cellular Longevity, vol. 2021, Article ID 5584447, 22 pages, 2021. <https://doi.org/10.1155/2021/5584447>
3. PubMed ID: -, Zhang Tao, Xiaoqing Zhou, Yan Zhang, Wenfeng Pu, Yi Yang, Fuxia Wei, Qian Zhou, Lin Zhang, Zhonghan Du, Ji Wu, "Xi Lei San Attenuates Dextran Sulfate Sodium-Induced Colitis in Rats and TNF-alpha-Stimulated Colitis in CACO2 Cells: Involvement of the NLRP3 Inflammasome and Autophagy", Mediators of Inflammation, vol. 2021, Article ID 1610251, 12 pages, 2021. <https://doi.org/10.1155/2021/1610251>

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