

Basic Information

Product Name	Anti-P62/SQSTM1 Antibody (Clone#3H11)	
Gene Name	SQSTM1	
Source	Mouse	
Isotype	IgG2a	
Species Reactivity	human, mouse, rat	
Tested Application	WB, IHC, ICC/IF, FCM	
Contents	200ug/ml antibody with PBS , 0.02% NaN ₃ , 1mg BSA and 50% glycerol.	
Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human SQSTM1/p62 (69-96aa DEDGDLVAFSSDEELTMAMSYVKDDIFR), identical to the related mouse and rat sequences.	
concentration	200ug/ml	
Purification	protein G purified.	
Observed MW	62KD	
Dilution Ratios	Western blot(WB): 1:500-2000 Immunohistochemistry in paraffin section (IHC): 1:50-400 Immunocytochemistry/Immunofluorescence (ICC/IF): 1:50-400 Flow cytometry (FCM): 1-3 µg/1x10 ⁶ cells (Boiling the paraffin sections in 10mM citrate buffer,pH6.0,or PH8.0 EDTA repair liquid for 20 mins is required for the staining of formalin/paraffin sections.) Optimal working dilutions must be determined by end user.	

Storage

12 months from date of receipt, -20°C as supplied. 6 months 2 to 8°C after reconstitution. Avoid repeated freezing and thawing.

Background Information

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.

Reference

Anti-P62/SQSTM1 Antibody (Clone#3H11)被引用在1文献中。

Selected Validation Data

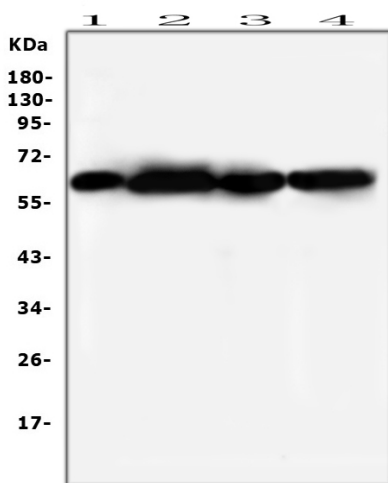


Figure 1. Western blot analysis of SQSTM1 using anti-SQSTM1 antibody (M00300-1).

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 50ug of sample under reducing conditions.

Lane 1: human CCRF-CEM whole cell lysates

Lane 2: human HepG2 whole cell lysates

Lane 3: human HT1080 whole cell lysates

Lane 4: human SW620 whole cell lysates

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA 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 µg/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 62KD. The expected band size for SQSTM1 is at 62KD.

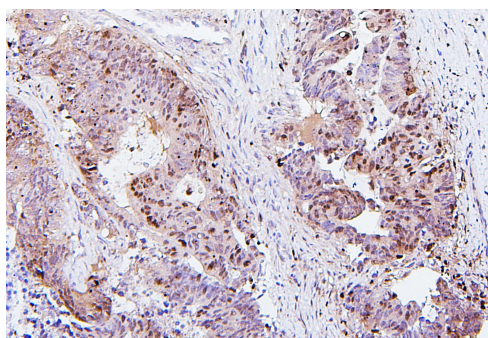


Figure 3. IHC analysis of SQSTM1 using anti-SQSTM1 antibody (M00300-1).

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 µg/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.

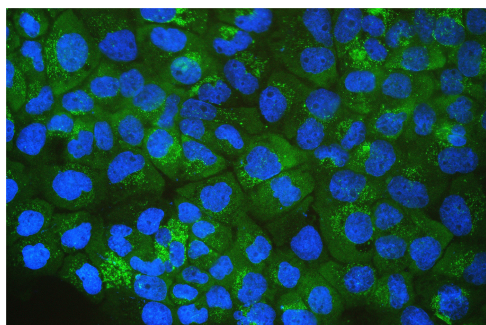


Figure 8. IF analysis of SQSTM1 using anti- SQSTM1 antibody (M00300-1)

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 tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2µg/mL mouse anti- SQSTM1 Antibody (M00300-1) overnight at 4°C. DyLight488 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.

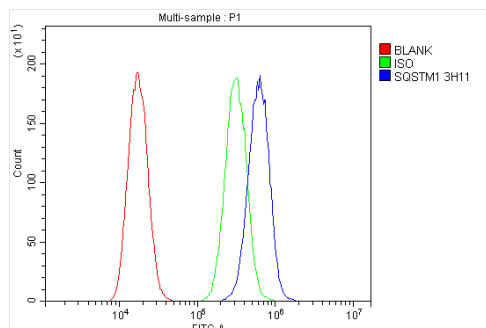


Figure 9. Flow Cytometry analysis of A549 cells using anti-SQSTM1 antibody (M00300-1).

Overlay histogram showing A549 cells stained with M00300-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-SQSTM1 Antibody (M00300-1, 1µg/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10µg/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1µg/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.