# abcam

## Product datasheet

## Alexa Fluor® 647 Anti-SQSTM1 / p62 antibody [EPR4844] ab194721





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#### Overview

**Product name** Alexa Fluor® 647 Anti-SQSTM1 / p62 antibody [EPR4844]

**Description** Alexa Fluor® 647 Rabbit monoclonal [EPR4844] to SQSTM1 / p62

**Host species** Rabbit

Conjugation Alexa Fluor® 647. Ex: 652nm, Em: 668nm

**Tested applications** Suitable for: Flow Cyt (Intra), ICC/IF

**Species reactivity** Reacts with: Human

Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. **Immunogen** 

Positive control ICC/IF: MCF7, HeLa cells (untreated and chloroquine-treated), HAP1 cells (HAP1-SQSTM1

knockout cells used as a negative cell line). Flow Cyt (intra): MCF7.

**General notes** This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

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### **Properties**

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle. Store In the Dark.

Storage buffer pH: 7.40

Preservative: 0.02% Sodium azide

Constituents: 30% Glycerol (glycerin, glycerine), 1% BSA, PBS

Purity Protein A purified

Clonality Monoclonal
Clone number EPR4844

**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our Abpromise quarantee covers the use of ab194721 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/500.  ab199093 - Rabbit monoclonal lgG (Alexa Fluor® 647), is suitable for use as an isotype control with this antibody.
ICC/IF		1/100 - 1/500.

## **Target**

**Function** Adapter protein which binds ubiquitin and may regulate the activation of NFKB1 by TNF-alpha,

nerve growth factor (NGF) and interleukin-1. May play a role in titin/TTN downstream signaling in muscle cells. May regulate signaling cascades through ubiquitination. Adapter that mediates the interaction between TRAF6 and CYLD (By similarity). May be involved in cell differentiation,

apoptosis, immune response and regulation of K(+) channels.

**Tissue specificity** Ubiquitously expressed.

Involvement in disease Defects in SQSTM1 are a cause of Paget disease of bone (PDB) [MIM:602080]. PDB is a

metabolic bone disease affecting the axial skeleton and characterized by focal areas of increased and disorganized bone turn-over due to activated osteoclasts. Manifestations of the

disease include bone pain, deformity, pathological fractures, deafness, neurological

complications and increased risk of osteosarcoma. PDB is a chronic disease affecting 2 to 3% of

the population above the age of 40 years.

Sequence similarities Contains 1 OPR domain.

Contains 1 UBA domain.
Contains 1 ZZ-type zinc finger.

#### **Domain**

The UBA domain binds specifically 'Lys-63'-linked polyubiquitin chains of polyubiquitinated substrates. Mediates the interaction with TRIM55.

The OPR domain mediates homooligomerization and interactions with PRKCZ, PRKCI, MAP2K5 and NBR1.

The ZZ-type zinc finger mediates the interaction with RIPK1.

Post-translational

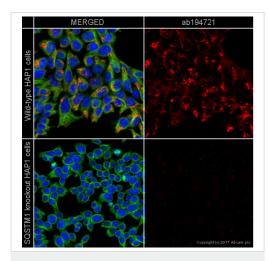
Cellular localization

modifications

Phosphorylated. May be phosphorylated by PRKCZ (By similarity). Phosphorylated in vitro by TTN.

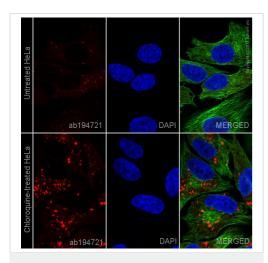
Cytoplasm. Late endosome. Nucleus. Sarcomere (By similarity). In cardiac muscles localizes to the sarcomeric band (By similarity). Localizes to late endosomes. May also localize to the nucleus. Accumulates in neurofibrillary tangles and in Lewy bodies of neurons from individuals with Alzheimer and Parkinson disease respectively. Enriched in Rosenthal fibers of pilocytic astrocytoma. In liver cells, accumulates in Mallory bodies associated with alcoholic hepatitis, Wilson disease, indian childhood cirrhosis and in hyaline bodies associated with hepatocellular carcinoma.

### **Images**



Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 647 Anti-SQSTM1 / p62 antibody [EPR4844] (ab194721) ab194721 staining SQSTM1 in wild-type HAP1 cells (top panel) and SQSTM1 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab194721 at 1/500 (shown in red) and <a href="mailto:ab195887">ab195887</a> at 1/250 dilution (shown in green). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 647 Anti-SQSTM1 / p62 antibody [EPR4844] (ab194721)

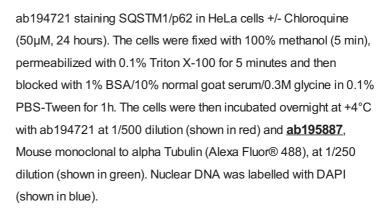
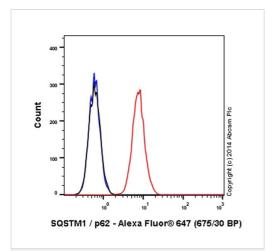


Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

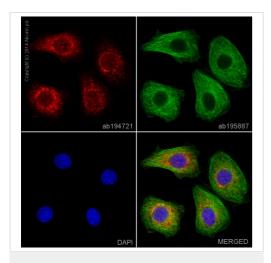


Flow Cytometry (Intracellular) - Alexa Fluor® 647 Anti-SQSTM1 / p62 antibody [EPR4844] (ab194721)

Overlay histogram showing MCF7 cells stained with ab194721 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab194721, 1/500 dilution) for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) Alexa Fluor® 647 used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a solid-state 25mW red diode laser (635 nm) and 675/30 bandpass filter.

This antibody gave a positive signal in MCF7 fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 647 Anti-SQSTM1 / p62 antibody [EPR4844] (ab194721)

ab194721 staining SQSTM1/p62 in MCF7 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab194721 at 1/100 dilution (shown in red) and ab195887, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor<sup>®</sup> 488, shown in green) at 1/167 dilution, overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



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