abcam

Product datasheet

Anti-SIRP alpha antibody [EPR22930-163] ab260039

Recombinant RabMAb

2 References 7 Images

Overview

Product name Anti-SIRP alpha antibody [EPR22930-163]

Description Rabbit monoclonal [EPR22930-163] to SIRP alpha

Host species Rabbit

Tested applications Suitable for: ICC/IF, IHC-P, WB, Flow Cyt

Unsuitable for: IP

Reacts with: Human Species reactivity

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Human brain, U-937, SK-MEL-28 and SK-MEL-2 lysates. IHC-P: Human cerebrum and

spleen tissues. ICC/IF: SK-MEL-28 cells. Flow Cyt: THP-1 cells.

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**.

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 long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number EPR22930-163 Isotype ΙgG

Applications

The Abpromise guarantee

Our Abpromise quarantee covers the use of ab260039 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
ICC/IF		1/250.
IHC-P		1/500.
WB		1/1000. Predicted molecular weight: 55 kDa.
Flow Cyt		1/600.

Application notes

Is unsuitable for IP.

Target

u			

Immunoglobulin-like cell surface receptor for CD47. Acts as docking protein and induces translocation of PTPN6, PTPN11 and other binding partners from the cytosol to the plasma membrane. Supports adhesion of cerebellar neurons, neurite outgrowth and glial cell attachment. May play a key role in intracellular signaling during synaptogenesis and in synaptic function (By similarity). Involved in the negative regulation of receptor tyrosine kinase-coupled cellular responses induced by cell adhesion, growth factors or insulin. Mediates negative regulation of phagocytosis, mast cell activation and dendritic cell activation. CD47 binding prevents maturation of immature dendritic cells and inhibits cytokine production by mature dendritic cells.

Tissue specificity

Ubiquitous. Highly expressed in brain. Detected on myeloid cells, but not T-cells. Detected at lower levels in heart, placenta, lung, testis, ovary, colon, liver, small intestine, prostate, spleen, kidney, skeletal muscle and pancreas.

Sequence similarities

Contains 2 lg-like C1-type (immunoglobulin-like) domains. Contains 1 lg-like V-type (immunoglobulin-like) domain.

Post-translational

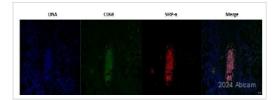
N-glycosylated.

modifications

Phosphorylated on tyrosine residues in response to stimulation with EGF, growth hormone, insulin and PDGF. Dephosphorylated by PTPN11.

Cellular localization Membrane.

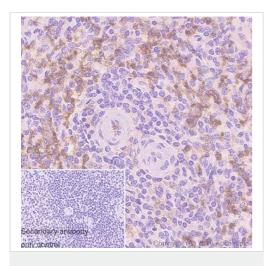
Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SIRP alpha antibody
[EPR22930-163] (ab260039)

This image is courtesy of an Abreview submitted by Christos Efstathiou

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of formaldehyde-fixed human lung tissue staining with ab260039 at 1/250 dilution. Sections were cut at 2 µm from lung tissues and mounted on poly-L-lysin coated slides. Samples were incubated with the primary antibody with BSA 5% in PBS for 16 hours at 25°C. Then, the slides were washed in PBS pH 7.4, incubated with secondary antibody Alexa Fluor™ 568 Goat antirabbit lgG(H+L) at 1/250 dilution for 30 min at room temperature. Blocking was done using 5% BSA for 20 minutes at 25°C. The second step was performed using primary antibody to CD68 for 1h at room temperature. Then, the slides were washed in PBS pH 7.4, incubated with the appropriate anti mouse secondary antibody 1/250 dilution for 30 min and the DNA was counterstained with Hoechst 33342 at 1µg/ml for 30 min at room temperature. After final washes with PBS, coverslips were mounted on the top of the samples using homemade Mowiol mounting medium.

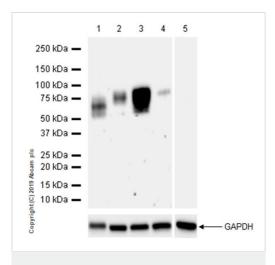


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SIRP alpha antibody
[EPR22930-163] (ab260039)

Immunohistochemical analysis of paraffin-embedded human spleen tissue labeling SIRP alpha with ab260039 at 1/500 followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on the human spleen is observed. The section was incubated with ab260039 for 15 mins at room temperature. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin.

Secondary antibody only control: Ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Western blot - Anti-SIRP alpha antibody [EPR22930-163] (ab260039)

All lanes: Anti-SIRP alpha antibody [EPR22930-163] (ab260039) at 1/1000 dilution

Lane 1: Human brain lysate

Lane 2: U-937 (human histiocytic lymphoma monocyte) whole cell

Lane 3: SK-MEL-28 (human malignant melanoma) whole cell

lysate

Lane 4: SK-MEL-2 (human skin malignant melanoma) whole cell

Lane 5: Jurkat (human T-cell leukemia lymphocyte) whole cell

lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 55 kDa Observed band size: 85 kDa

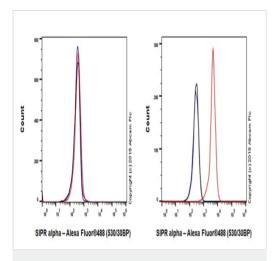
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

There are many forms of SIRP alpha because of different glycosylation and phosphotyrosine modification.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 9712903).

Negative control: Jurkat (PMID: 17982459).

Exposure time: 3 minutes

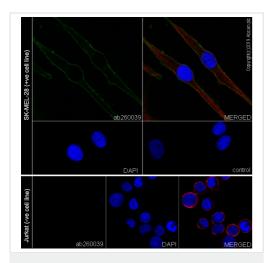


Flow Cytometry - Anti-SIRP alpha antibody [EPR22930-163] (ab260039)

Flow cytometric analysis of Jurkat (Human T cell leukemia T lymphocyte, Left) / THP-1 (Human monocytic leukemia monocyte, Right) cells labeling SIRP alpha with ab260039 at 1\600 compared with a Rabbit monoclonal IgG (ab172730) / Black isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 was used as the secondary antibody.

Negative control: Jurkat.

Gated on viable cells.



Immunocytochemistry/ Immunofluorescence - Anti-SIRP alpha antibody [EPR22930-163] (ab260039)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized SK-MEL-28 (human malignant melanoma melanoma) cells labeling SIRP alpha with ab260039 at 1/250 dilution, followed by ab150077 AlexaFluor[®] 488 Goat anti-Rabbit secondary antibody at 1/1000 dilution (Green). Confocal image showing membranous staining in SK-MEL-28 cells

Negative control: Jurkat (PMID: 17982459) is observed.

ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: <u>ab150077</u> AlexaFluor[®]488 Goat anti-Rabbit secondary at 1/1000 dilution.

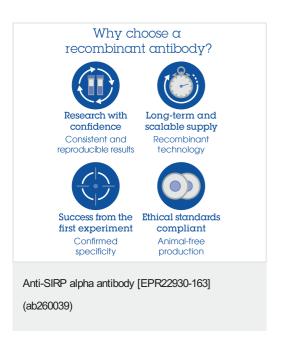


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SIRP alpha antibody
[EPR22930-163] (ab260039)

Immunohistochemical analysis of paraffin-embedded human cerebrum tissue labeling SIRP alpha with ab260039 at 1/500 followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on the human cerebrum (PMID: 9062191) is observed. The section was incubated with ab260039 for 15 mins at room temperature. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin.

Secondary antibody only control: Ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



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