abcam

ab191419

Anti-SIRP alpha antibody [EPR16264]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR16264
Purification technique	Affinity purification Protein A
Concentration	0.31 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Rat
Dilution info	1/1000 - 1/2000
Notes	-

Species	Mouse

Dilution info	1/1000 - 1/2000
Notes	-

Species	Human
Dilution info	1/1000 - 1/2000
Notes	-

ICC/IF

Tested

Species	Rat
Dilution info	1/500
Notes	-

Expected

Species	Mouse, Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/50
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information SIRPA

Function	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. Plays a role in antiviral immunity and limits new world arenavirus infection by decreasing virus internalization (By similarity). Receptor for THBS1 (PubMed:24511121). Interaction with THBS1 stimulates phosphorylation of SIRPA (By similarity). In response to THBS1, involved in ROS signaling in non-phagocytic cells, stimulating NADPH oxidase-derived ROS production (PubMed:24511121).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

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

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	SIRP alpha also known as CD172a is a transmembrane receptor protein with a mass ranging between 70-110 kDa due to glycosylation. This protein extensively expresses on the surface of myeloid cells neurons and a subset of T-cells and is part of the immunoglobulin superfamily. SIRP alpha interacts with its ligand CD47 a widely expressed glycoprotein involved in immune response regulation. Its mechanical action primarily involves signal regulation through the recruitment of SHP-1 and SHP-2 two cytoplasmic tyrosine phosphatases.
Biological function summary	SIRP alpha functions significantly in the regulation of phagocytosis acting as a "don't eat me" signal to macrophages upon binding with CD47. It does not act alone; rather it is part of a complex that recruits SHP-1 and SHP-2 leading to inhibition of dephosphorylation activities essential for engulfment processes. This regulatory mechanism is important for maintaining cellular homeostasis ensuring that healthy cells are not mistakenly destroyed by the immune system.
Pathways	SIRP alpha plays an important role in the innate immune pathways involving the regulation of phagocytosis and cell-cell adhesion. Particularly it fits into the immune checkpoint pathways where it interacts closely with proteins like CD47 and plays a role in the interaction between the immune system and cancer cells. Through these pathways SIRP alpha helps maintain balance in the immune response allowing for the recognition of self versus non-self therefore preventing autoimmunity while facilitating the clearance of pathogens.
Associated diseases and disorders	SIRP alpha is implicated in cancer and autoimmune diseases. In cancer its interaction with CD47 allows tumor cells to escape phagocytosis promoting tumor survival and growth. In autoimmune disorders dysregulated SIRP alpha expression or signaling could miscommunicate immune signals leading to the destruction of healthy tissue. Understanding the link between SIRP alpha and these conditions can reveal potential targets for therapeutic development especially using inhibitors or modulators targeting the SIRP alpha-CD47 interaction.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

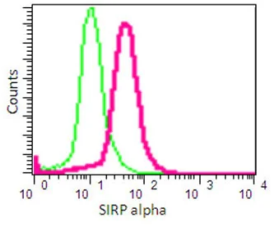
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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7 product images

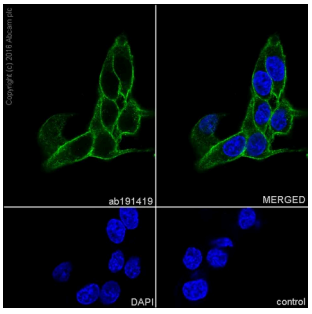


Flow Cytometry (Intracellular) - Anti-SIRP alpha antibody [EPR16264] (ab191419)

SIRP alpha Flow Cytometry (Intracellular) staining using rabbit Anti-SIRP alpha antibody

Intracellular flow cytometric analysis of THP1 cells (2% paraformaldehyde-fixed) labeling SIRP alpha with ab191419 at 1/50 dilution (red) or a Rabbit monoclonal IgG (negative) (green), followed by Goat anti rabbit IgG (FITC) secondary at 1/150 dilution

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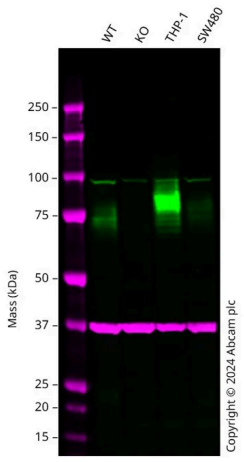


Immunocytochemistry/ Immunofluorescence - Anti-SIRP alpha antibody [EPR16264] (ab191419)

SIRP alpha Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-SIRP alpha antibody

Immunocytochemistry/ Immunofluorescence analysis of C6 (rat glial tumor glial cell) labeling SIRP alpha with ab191419 at 1/500. ab150077 Alexa Fluor® 488 Goat anti-Rabbit at 1/1000 was used as the secondary antibody. Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% tritonX-100. DAPI was used to stain nuclei blue.

Confocal image showing membranous staining on C6 cell line.



Western blot - Anti-SIRP alpha antibody [EPR16264] (ab191419)

SIRP alpha Western blot staining using rabbit Anti-SIRP alpha antibody

Western blot: Anti-SIRPA antibody [EPR16264] (ab191419) staining at 1/1500 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab191419 was shown to bind specifically to SIRPA. A band was observed at 75-98 kDa in wild-type A549 cell lysates with no signal observed at this size in SIRPA knockout cell line. To generate this image, wild-type and SIRPA knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-SIRP alpha antibody [EPR16264] (ab191419) at 1/1500 dilution

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
Western blot - Human SIRPA knockout A549 cell line (ab287760) at 20 µg

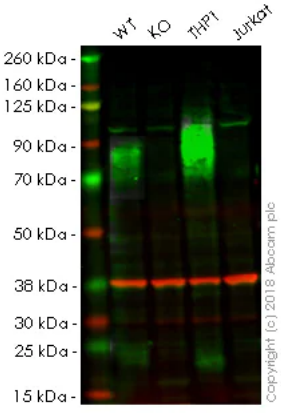
Lane 3:
THP-1 cell lysate at 20 µg

Lane 4:
SW480 cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.



Western blot - Anti-SIRP alpha antibody [EPR16264] (ab191419)

SIRP alpha Western blot staining using rabbit Anti-SIRP alpha antibody

Lanes 1 - 4: Merged signal (red and green). Green - ab191419 observed at 55 kDa. Red - loading control, ab9484, observed at 37 kDa.
ab191419 was shown to recognize SIRP alpha in wild-type HAP1 cells as signal was lost at the expected MW in SIRPA knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and SIRPA knockout samples were subjected to SDS-PAGE. ab191419 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-SIRP alpha antibody [EPR16264] (ab191419) at 1/1000 dilution

Lane 1:
Wild-type HAP1 whole cell lysate at 20 µg

Lane 2:

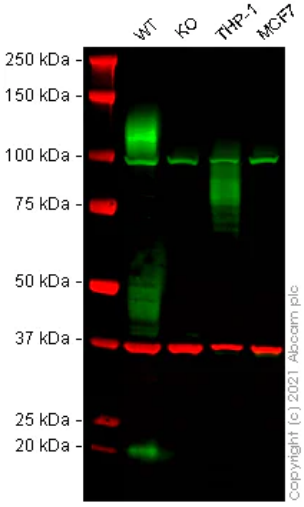
SIRPA knockout HAP1 whole cell lysate at 20 µg

Lane 3:
THP1 whole cell lysate at 20 µg

Lane 4:
Jurkat whole cell lysate (negative control) at 20 µg

Predicted band size: 55 kDa

Observed band size: 55 kDa



Western blot - Anti-SIRP alpha antibody [EPR16264] (ab191419)

SIRP alpha Western blot staining using rabbit Anti-SIRP alpha antibody

False colour image of Western blot: Anti-SIRP alpha antibody [EPR16264] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab191419 was shown to bind specifically to SIRP alpha. A band was observed at 100-140 kDa (mouse SIRPA, isoform 1) & 40-50 kDa (mouse SIRPA, isoform 2), in wild-type RAW 264.7 cell lysates (band observed at 70-100 kDa in THP-1 is Human SIRPA) with no signal observed at this size in SIRPA knockout cell line ab281618 (knockout cell lysate ab282969). To generate this image, wild-type and SIRPA knockout RAW 264.7 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.

Glycosylation level (~65-120 kDa) of SIRPα is different in various tissues (PMID: 18051954).

observed band: 100-140 kDa (mouse SIRPA, isoform 1) & 40-50 kDa (mouse SIRPA, isoform 2)

All lanes:
Western blot - Anti-SIRP alpha antibody [EPR16264] (ab191419) at 1/1000 dilution

Lane 1:
Wild-type RAW 264.7 cell lysate at 20 µg

Lane 2:
SIRPA knockout RAW 264.7 cell lysate at 20 µg

Lane 2:
Western blot - Mouse SIRPA knockout RAW 264.7 cell line (ab281618)

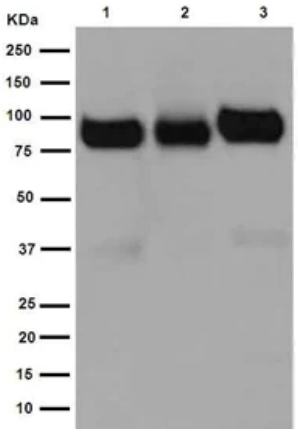
Lane 3:
THP-1 cell lysate at 20 µg

Lane 4:
MCF7 cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 55 kDa

Observed band size: 100-140 kDa



Western blot - Anti-SIRP alpha antibody [EPR16264] (ab191419)

All lanes:
Western blot - Anti-SIRP alpha antibody [EPR16264] (ab191419) at 1/1000 dilution

Lane 1:
C6 cell lysate at 10 µg

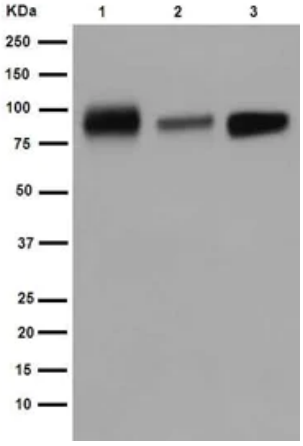
Lane 2:
PC12 cell lysate at 10 µg

Lane 3:
NIH 3T3 cell lysate at 10 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 55 kDa



Western blot - Anti-SIRP alpha antibody [EPR16264] (ab191419)

All lanes:
Western blot - Anti-SIRP alpha antibody [EPR16264] (ab191419) at 1/5000 dilution

Lane 1:
THP1 cell lysate at 20 µg

Lane 2:
SW480 cell lysate at 20 µg

Lane 3:
Human fetal brain lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 55 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.