abcam

Product datasheet

Anti-SIRP alpha antibody [EPR16264] ab191419

敲除验证 重组 RabMAb

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概述

产品名称	Anti-SIRP alpha抗体[EPR16264]
描述	兔单克隆抗体[EPR16264] to SIRP alpha
宿主	Rabbit
经测试应用	**适用于:** ICC/IF, Flow Cyt (Intra), WB
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: THP1, SW480, C6, PC12, NIH 3T3 and Human fetal brain lysates. Flow Cyt (intra): THP1 cells. ICC/IF: C6 cells
常规说明	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**.

性能

形式	Liquid
存放说明	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.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR16264
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab191419于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF		1/500.
Flow Cyt (Intra)		1/50. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		1/1000 - 1/2000. Detects a band of approximately 75-110 kDa (predicted molecular weight: 55 kDa).

靶标

功能 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.

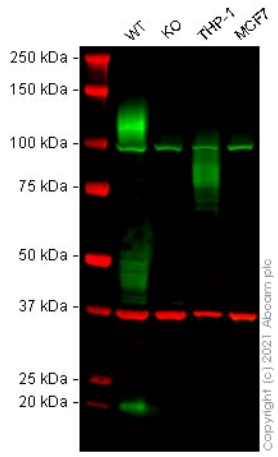
组织特异性 Ubiquitous. Highly expressed in brain. Detected on myeloid cells, but not T-cells. Detected at lower levels in heart, placenta, lung, testis, ovary, colon, liver, small intestine, prostate, spleen, kidney, skeletal muscle and pancreas.

序列相似性 Contains 2 Ig-like C1-type (immunoglobulin-like) domains.
Contains 1 Ig-like V-type (immunoglobulin-like) domain.

翻译后修饰 N-glycosylated.
Phosphorylated on tyrosine residues in response to stimulation with EGF, growth hormone, insulin and PDGF. Dephosphorylated by PTPN11.

细胞定位 Membrane.

图片



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

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

Lane 1 : Wild-type RAW 264.7 cell lysate
Lane 2 : SIRPA knockout RAW 264.7 cell lysate
Lane 3 : THP-1 cell lysate
Lane 4 : MCF7 cell lysate

Lysates/proteins at 20 µg per lane.

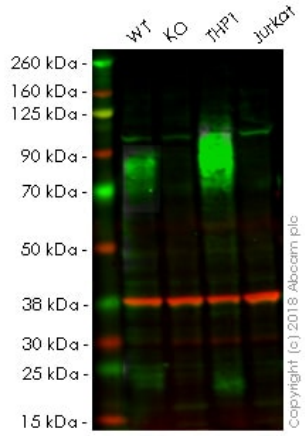
Performed under reducing conditions.

Predicted band size: 55 kDa
Observed band size: 100-140 kDa

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).



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

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

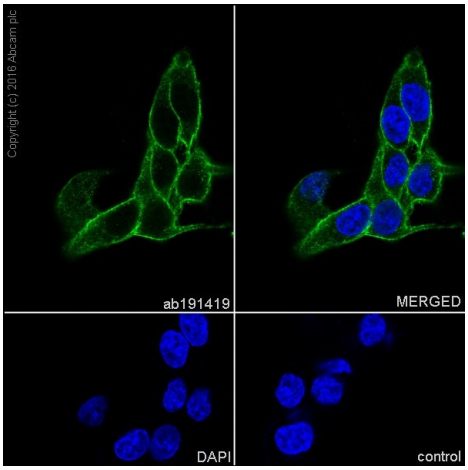
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : SIRPA knockout HAP1 whole cell lysate
Lane 3 : THP1 whole cell lysate
Lane 4 : Jurkat whole cell lysate (negative control)

Lysates/proteins at 20 µg per lane.

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

Lanes 1 - 4: Merged signal (red and green). Green - ab191419 observed at 55 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

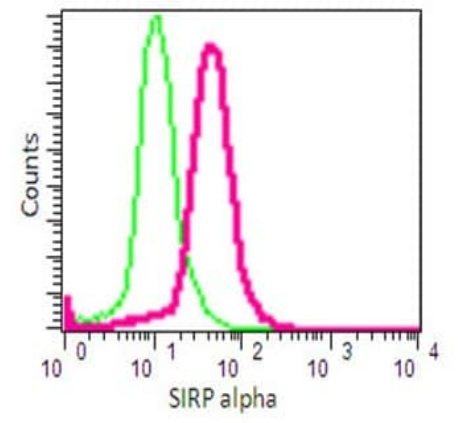
ab191419 was shown to recognize SIRP alpha in wild-type HAP1 cells as signal was lost at the expected MW in SIRPA knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and SIRPA knockout samples were subjected to SDS-PAGE. Ab191419 and **ab9484** (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.



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

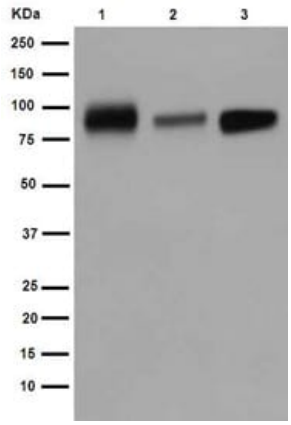
Immunocytochemistry/ Immunofluorescence analysis of C6 (rat glial tumor glial cell) labeling SIRP alpha with ab191419 at 1/500. **ab150077** Alexa Fluor® 488 Goat anti-Rabbit at 1/1000 was used as the secondary antibody. Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% tritonX-100.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

Confocal image showing membranous staining on C6 cell line.



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

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



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

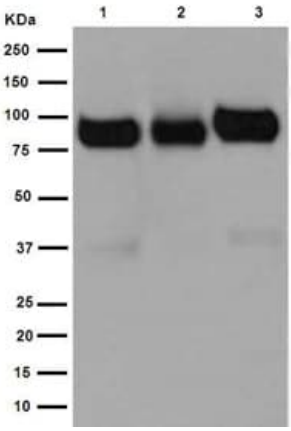
All lanes : Anti-SIRP alpha antibody [EPR16264] (ab191419) at 1/5000 dilution

Lane 1 : THP1 cell lysate
Lane 2 : SW480 cell lysate
Lane 3 : Human fetal brain lysate

Lysates/proteins at 20 µg per lane.

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)

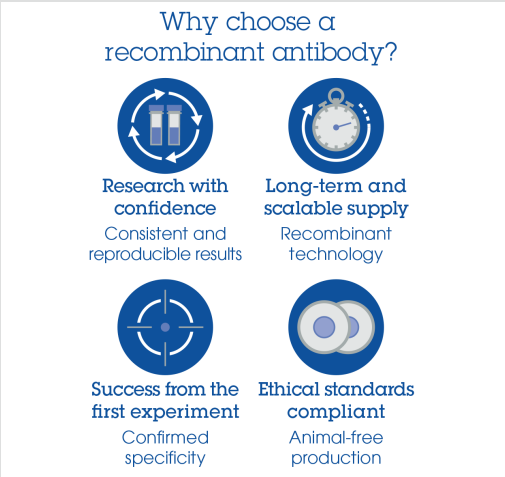
All lanes : Anti-SIRP alpha antibody [EPR16264] (ab191419) at 1/1000 dilution

Lane 1 : C6 cell lysate
Lane 2 : PC12 cell lysate
Lane 3 : NIH 3T3 cell lysate

Lysates/proteins at 10 µg per lane.

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

Predicted band size: 55 kDa



Anti-SIRP alpha antibody [EPR16264] (ab191419)

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