abcam

## Product datasheet

# Mouse SIRPA knockout RAW 264.7 cell lysate ab282969

3 Images

### Overview

| | |
|---|---|
| **Product name** | Mouse SIRPA knockout RAW 264.7 cell lysate |
| **Product overview** | Knockout cell lysate achieved by CRISPR/Cas9. |
| **Parental Cell Line** | RAW 264.7 |
| **Organism** | Mouse |
| **Mutation description** | Knockout achieved by using CRISPR/Cas9, Homozygous: 35 bp deletion in exon 6 |
| **Passage number** | <20 |
| **Knockout validation** | Sanger Sequencing, Western Blot (WB) |
| **Reconstitution notes** | To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT.<br>**Usage of SDS sample buffer is not recommended with these lyophilized lysates.* |
| **Notes** | **Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared.<br><br>**User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage.<br><br>Access thousands of knockout cell lysates, generated from commonly used cancer cell lines.<br>**See here for more information on knockout cell lysates.**<br><br>Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.<br>It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.<br><br>This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**. |

### Properties

**Storage instructions** Store at -80°C. Please refer to protocols.

| Components | 1 kit |
|---|---|
| ab283113 - Mouse SIRPA knockout RAW 264.7 cell lysate | 1 x 100µg |
| ab277354 - Mouse wild-type RAW 264.7 cell lysate | 1 x 100µg |

**Cell type** leukaemic monocyte macrophage

**Disease** Carcinoma

**Gender** Male

## Target

**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.

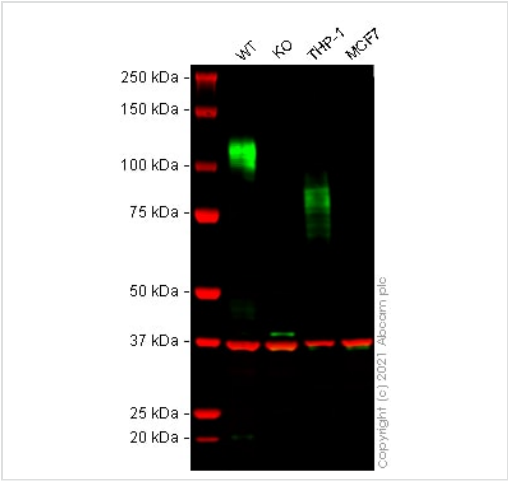
**Tissue specificity** Ubiquitous. Highly expressed in brain. Detected on myeloid cells, but not T-cells. Detected at lower levels in heart, placenta, lung, testis, ovary, colon, liver, small intestine, prostate, spleen, kidney, skeletal muscle and pancreas.

**Sequence similarities** Contains 2 Ig-like C1-type (immunoglobulin-like) domains.
Contains 1 Ig-like V-type (immunoglobulin-like) domain.

**Post-translational modifications** N-glycosylated.
Phosphorylated on tyrosine residues in response to stimulation with EGF, growth hormone, insulin and PDGF. Dephosphorylated by PTPN11.

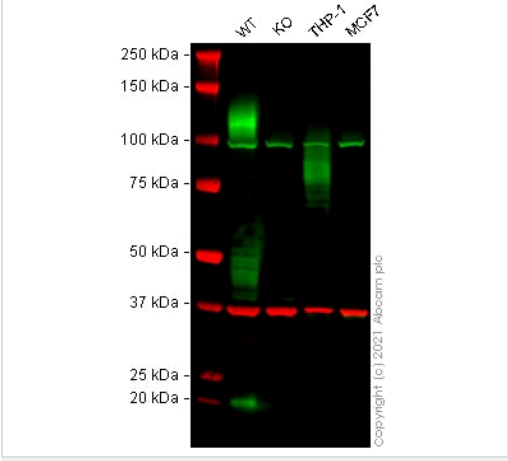
**Cellular localization** Membrane.

## Images




Western blot - Mouse SIRPA knockout RAW 264.7 cell lysate (ab282969)

**Lane 1:** Wild-type RAW 264.7 cell lysate 20 µg
**Lane 2:** RAW 264.7 cell lysate 20 µg
**Lane 3:** THP-1 cell lysate 20 µg
**Lane 4:** MCF7 cell lysate 20 µg
False colour image of Western blot: Anti-SIRPA antibody 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, the antibody was shown to bind specifically to SIRPA. A band was observed at 100-140 kDa (mouse SIRPA, isoform 1), 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.




Western blot - Mouse SIRPA knockout RAW 264.7 cell lysate (ab282969)

**Lane 1:** Wild-type RAW 264.7 cell lysate 20 µg
**Lane 2:** SIRPA knockout RAW 264.7 cell lysate 20 µg
**Lane 3:** THP-1 cell lysate 20 µg
**Lane 4:** MCF7 cell lysate 20 µg
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.


35 bp deletion in exon 6

Mouse SIRPA knockout RAW 264.7 cell lysate



## Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

## Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors