abcam

Product datasheet

Anti-SHIP-1 antibody [EP378Y] ab45142

Recombinant RabMAb

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Overview

Product name Anti-SHIP-1 antibody [EP378Y]

Description Rabbit monoclonal [EP378Y] to SHIP-1

Host species Rabbit

Tested applications Suitable for: ICC/IF, WB, IHC-P, IP, Flow Cyt (Intra)

Species reactivity Reacts with: Mouse, Human

Immunogen Synthetic peptide within Human SHIP-1 aa 1100 to the C-terminus (C terminal). The exact

sequence is proprietary.

Positive control WB: KM3, Daudi and Ramos cell lysates. IHC-P: Human and mouse spleen tissues. ICC/IF:

Jurkat and Raji cells. Flow Cyt (intra): Raji cells. IP: Daudi cell lysate.

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**.

Rat: We have preliminary internal testing data to indicate this antibody may not react with this

species. Please contact us for more information.

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.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EP378Y
Isotype IqG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab45142 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		1/150.
WB		1/1000. Detects a band of approximately 140 kDa (predicted molecular weight: 133 kDa). For unpurified use at 1/50000.
IHC-P		1/100 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
IP		1/20 - 1/50.
Flow Cyt (Intra)		1/20 - 1/50. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

Target

Function

Phosphatidylinositol (PtdIns) phosphatase that specifically hydrolyzes the 5-phosphate of phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P3) to produce PtdIns(3,4)P2, thereby negatively regulating the Pl3K (phosphoinositide 3-kinase) pathways. Acts as a negative regulator of B-cell antigen receptor signaling. Mediates signaling from the FC-gamma-RllB receptor (FCGR2B), playing a central role in terminating signal transduction from activating immune/hematopoietic cell receptor systems. Acts as a negative regulator of myeloid cell proliferation/survival and chemotaxis, mast cell degranulation, immune cells homeostasis, integrin alpha-llb/beta-3 signaling in platelets and JNK signaling in B-cells. Regulates proliferation of osteoclast precursors, macrophage programming, phagocytosis and activation and is required for endotoxin tolerance. Involved in the control of cell-cell junctions, CD32a signaling in neutrophils and modulation of EGF-induced phospholipase C activity. Key regulator of neutrophil migration, by governing the formation of the leading edge and polarization required for chemotaxis. Modulates FCGR3/CD16-mediated cytotoxicity in NK cells. Mediates the activin/TGF-beta-induced apoptosis through its Smad-dependent expression. May also hydrolyze PtdIns(1,3,4,5)P4, and could thus affect the levels of the higher inositol polyphosphates like InsP6.

Tissue specificity

Specifically expressed in immune and hematopoietic cells. Expressed in bone marrow and blood cells. Levels vary considerably within this compartment. Present in at least 74% of immature CD34+ cells, whereas within the more mature population of CD33+ cells, it is present in only 10% of cells. Present in the majority of T-cells, while it is present in a minority of B-cells (at protein level).

Sequence similarities

Belongs to the inositol-1,4,5-trisphosphate 5-phosphatase family.

Contains 1 SH2 domain.

Domain

The SH2 domain interacts with tyrosine phosphorylated forms of proteins such as SHC1 or PTPN11/SHP-2. It competes with that of GRB2 for binding to phosphorylated SHC1 to inhibit the Ras pathway. It is also required for tyrosine phosphorylation.

The NPXY sequence motif found in many tyrosine-phosphorylated proteins is required for the specific binding of the PID domain.

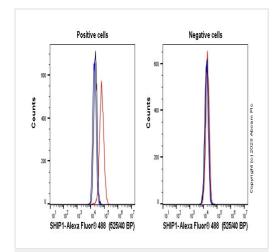
Post-translational modifications

Tyrosine phosphorylated by the members of the SRC family after exposure to a diverse array of extracellular stimuli such as cytokines, growth factors, antibodies, chemokines, integrin ligands and hypertonic and oxidative stress. Phosphorylated upon IgG receptor FCGR2B-binding.

Cellular localization

Cytoplasm. Membrane. Translocates to the plasma membrane when activated, translocation is probably due to different mechanisms depending on the stimulus and cell type. Partly translocated via its SH2 domain which mediates interaction with tyrosine phosphorylated receptors such as the FC-gamma-RIIB receptor (FCGR2B) or CD16/FCGR3. Tyrosine phosphorylation may also participate to membrane localization.

Images



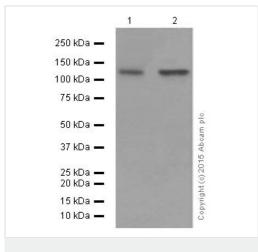
Flow Cytometry (Intracellular) - Anti-SHIP-1 antibody [EP378Y] (ab45142)

Flow cytometry overlay histogram showing left Raji positive cells and right negative MCF7 stained with ab45142 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10 μ g/ml human lgG and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (ab45142) (1x 10⁶ in 100 μ l at 0.008 μ g/ml (1/275000)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

sample (blue line) was also used as a control.



Western blot - Anti-SHIP-1 antibody [EP378Y] (ab45142)

All lanes : Anti-SHIP-1 antibody [EP378Y] (ab45142) at 1/5000 dilution (purified)

Lane 1 : Daudi cell lysate
Lane 2 : Ramos cell lysate

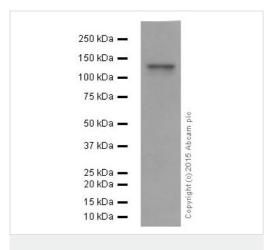
Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Peroxidase-conjugated goat anti-rabbit lgG, (H+L) at 1/1000 dilution

Predicted band size: 133 kDa **Observed band size:** 140 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-SHIP-1 antibody [EP378Y] (ab45142)

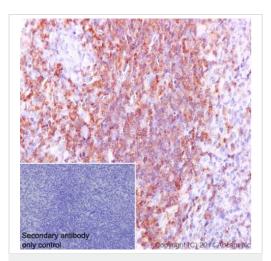
Anti-SHIP-1 antibody [EP378Y] (ab45142) at 1/1000 dilution (purified) + KM3 cell lysate at 20 µg

Secondary

Peroxidase-conjugated goat anti-rabbit lgG, (H+L) at 1/1000 dilution

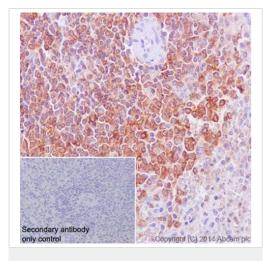
Predicted band size: 133 kDa **Observed band size:** 140 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



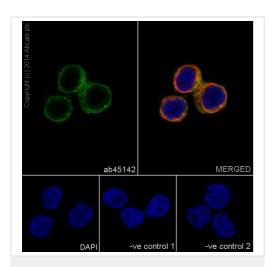
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SHIP-1 antibody
[EP378Y] (ab45142)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse spleen tissue labelling SHIP-1 with purified ab45142 at 1/500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. <u>ab97051</u>, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

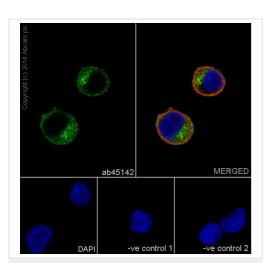


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SHIP-1 antibody
[EP378Y] (ab45142)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human spleen tissue labelling SHIP-1 with purified ab45142 at 1/500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. <u>ab97051</u>, a HRP-conjugated goat anti-rabbit lgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunocytochemistry/ Immunofluorescence - Anti-SHIP-1 antibody [EP378Y] (ab45142)



Immunocytochemistry/ Immunofluorescence - Anti-SHIP-1 antibody [EP378Y] (ab45142)

Immunocytochemistry/Immunofluorescence analysis of Raji cells labelling SHIP-1 with purified ab45142 at 1/150. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor[®] 488-conjugated goat anti-rabbit lgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse anti-tubulin (1/1000) and ab150120, an Alexa Fluor[®] 594-conjugated goat antimouse lgG (1/500) were also used.

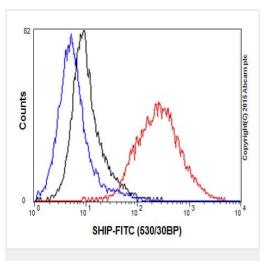
Control 1: primary antibody (1/150) and secondary antibody, **ab150120**, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/500).

Control 2: <u>ab7291</u> (1/1000) and secondary antibody, <u>ab150077</u>, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/500).

Immunocytochemistry/Immunofluorescence analysis of Jurkat cells labelling SHIP-1 with purified ab45142 at 1/150. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse anti-tubulin (1/1000) and ab150120, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/500) were also used.

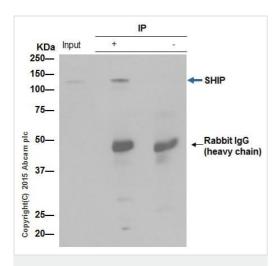
Control 1: primary antibody (1/150) and secondary antibody, **ab150120**, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/500).

Control 2: $\underline{ab7291}$ (1/1000) and secondary antibody, $\underline{ab150077}$, an Alexa Fluor® 488-conjugated goat anti-rabbit lgG (1/500).



Flow Cytometry (Intracellular) - Anti-SHIP-1 antibody [EP378Y] (ab45142)

Intracellular Flow Cytometry analysis of Raji cells labelling SHIP-1 with purified ab45142 at 1/50 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit lgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal lgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Immunoprecipitation - Anti-SHIP-1 antibody [EP378Y] (ab45142)

ab45142 (purified) at 1/20 immunoprecipitating SHIP-1 in Daudi whole cell lysate.

Lane 1 (input): Daudi whole cell lysate (10µg)

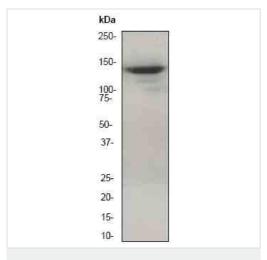
Lane 2 (+): ab45142 + Daudi whole cell lysate (10µg).

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab45142 in Daudi whole cell lysate.

For western blotting, a HRP-conjugated anti-rabbit lgG, specific to the non-reduced form of lgG was used as the secondary antibody (1/1500).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



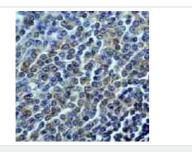
Western blot - Anti-SHIP-1 antibody [EP378Y] (ab45142)

Anti-SHIP-1 antibody [EP378Y] (ab45142) at 1/50000 dilution (unpurified) + Daudi cell lysate at 10 μg

Secondary

HRP-conjugated goat anti-rabbit IgG at 1/2000 dilution

Predicted band size: 133 kDa **Observed band size:** 140 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SHIP-1 antibody
[EP378Y] (ab45142)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human spleen tissue labelling SHIP-1 with unpurified ab45142 at a dilution of 1/100.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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