

Product datasheet

Anti-SHIP antibody [EP378Y] α b45142

Recombinant RabMAB

2 References 10 Images

Overview

Product name	Anti-SHIP antibody [EP378Y]
Description	Rabbit monoclonal [EP378Y] to SHIP
Host species	Rabbit
Tested applications	Suitable for: ICC/IF, WB, IHC-P, Flow Cyt, IP
Species reactivity	Reacts with: Mouse, Human
Immunogen	Synthetic peptide within Human SHIP 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: Raji cells. IP: Daudi cell lysate.
General notes	<p>Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.</p> <p>Our RabMAB[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab[®] patents</p> <p>We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.</p> <p>This product is a recombinant rabbit monoclonal antibody.</p>

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 IgG

Applications

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 .
Flow Cyt		1/20 - 1/50. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP		1/20 - 1/50.

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 PI3K (phosphoinositide 3-kinase) pathways. Acts as a negative regulator of B-cell antigen receptor signaling. Mediates signaling from the FC-gamma-R1IB 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-IIb/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.

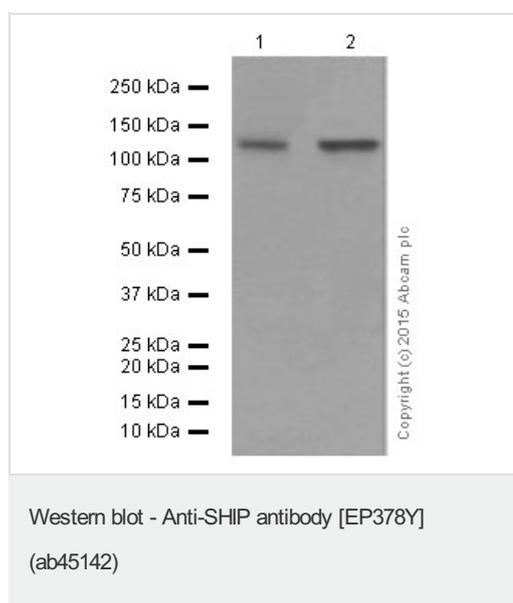
Post-translational modifications

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-R1IB receptor (FCGR2B) or CD16/FCGR3. Tyrosine phosphorylation may also participate to membrane localization.

Images



All lanes : Anti-SHIP 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.

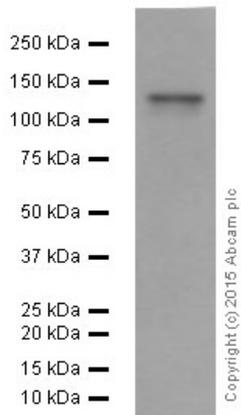
Secondary

All lanes : Peroxidase-conjugated goat anti-rabbit IgG, (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 antibody [EP378Y]
(ab45142)

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

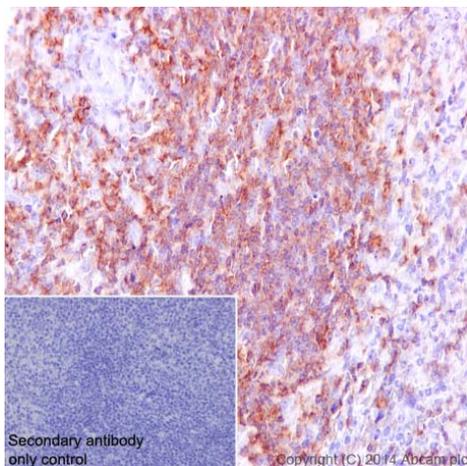
Secondary

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

Predicted band size: 133 kDa

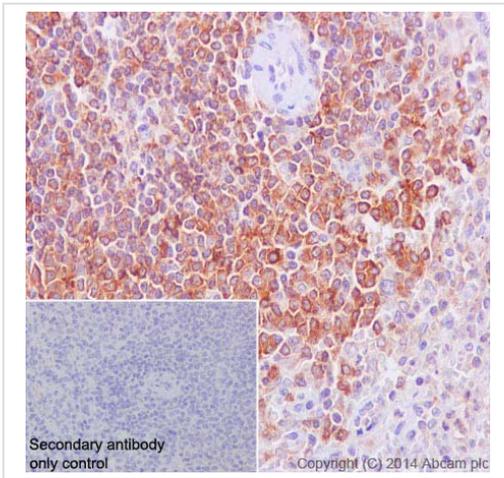
Observed band size: 140 kDa

Blocking and dilution buffer: 5% NFDm/TBST.



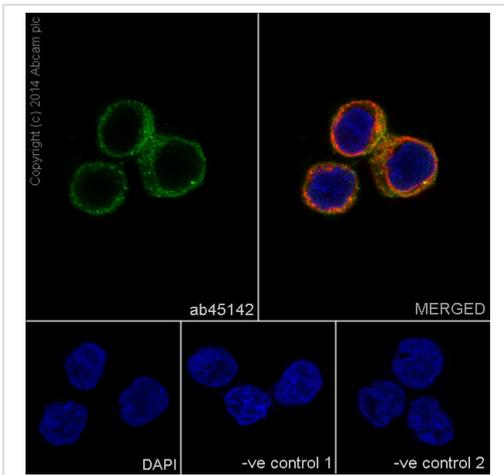
Immunohistochemistry (Formalin/PFA-fixed paraffin-
embedded sections) - Anti-SHIP antibody [EP378Y]
(ab45142)

Immunohistochemistry (Formalin/PFA-fixed
paraffin-embedded sections) analysis of
mouse spleen tissue labelling SHIP with
purified ab45142 at 1/500. Heat mediated
antigen retrieval was performed using
Tris/EDTA buffer pH 9. [ab97051](#), 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 paraffin-embedded sections) analysis of human spleen tissue labelling SHIP with purified ab45142 at 1/500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. [ab97051](#), 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 paraffin-embedded sections) - Anti-SHIP antibody [EP378Y] (ab45142)

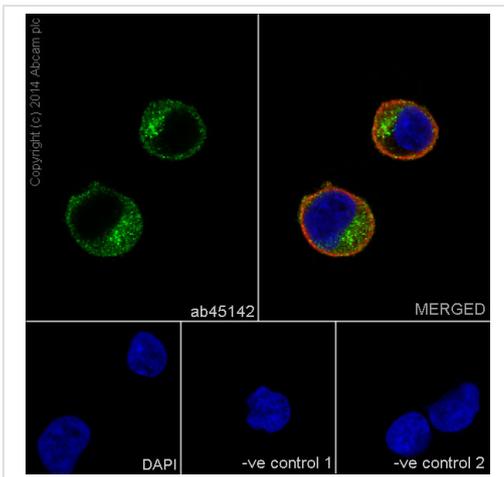


Immunocytochemistry/Immunofluorescence analysis of Raji cells labelling SHIP with purified ab45142 at 1/150. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. [ab150077](#), an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. 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.

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

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

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

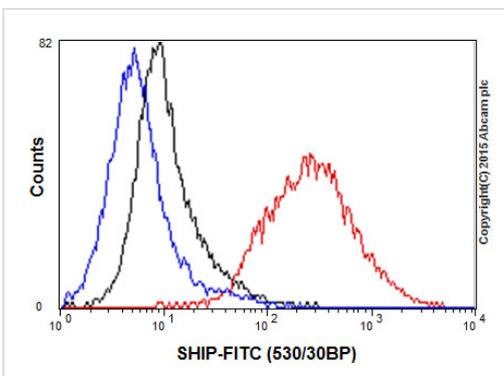


Immunocytochemistry/ Immunofluorescence - Anti-SHIP antibody [EP378Y] (ab45142)

Immunocytochemistry/Immunofluorescence analysis of Jurkat cells labelling SHIP 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.

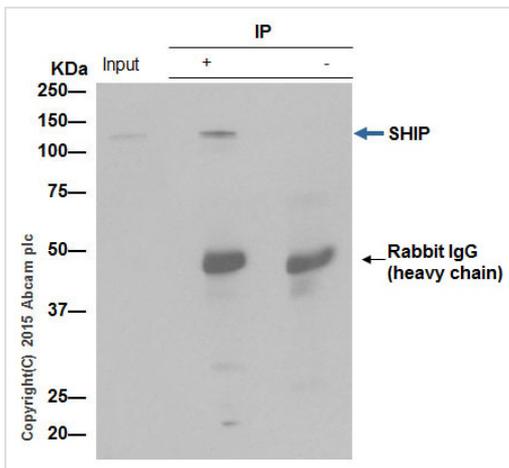
Control 1: primary antibody (1/150) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

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



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

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



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

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

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

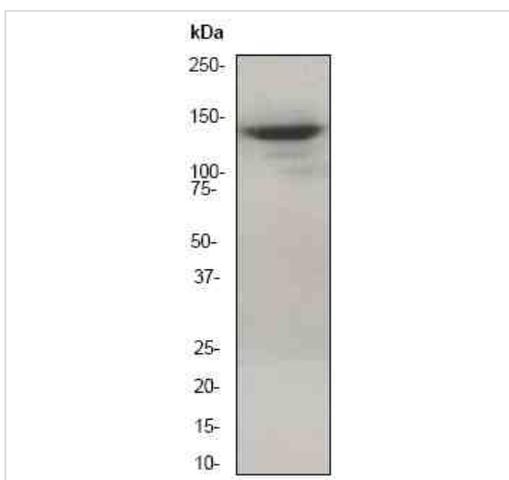
Lane 2 (+): ab45142 + Daudi whole cell lysate (10µg).

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

For western blotting, a HRP-conjugated anti-rabbit IgG, specific to the non-reduced form of IgG 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 antibody [EP378Y] (ab45142)

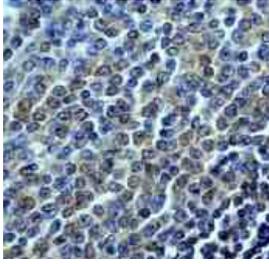
Anti-SHIP antibody [EP378Y] (ab45142) at 1/50000 dilution (unpurified) + Daudi cell lysate at 10 µg

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

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

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