

Product datasheet

Anti-SRC Family (phospho Y418) antibody [EP503Y] ab40660

Recombinant RabMAb

★★★★☆ 2 Abreviews 27 References 7 Images

Overview

Product name	Anti-SRC Family (phospho Y418) antibody [EP503Y]
Description	Rabbit monoclonal [EP503Y] to SRC Family (phospho Y418)
Host species	Rabbit
Specificity	ab40660 recognises Lyn phosphorylation on Tyrosine 396. BLAST analysis show 100% homology with Hck (pY410) and Lck (pY393), and 92% homology with Src (pY418), Fyn (pY419) and Yes (pY425) Therefore, the antibody may cross-react with these proteins.
Tested applications	Suitable for: WB, Flow Cyt, ICC/IF, ELISA
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide within Human SRC Family aa 350-450 (phospho Y418). The exact sequence is proprietary. Database link: P07948 (Peptide available as ab202645)
Positive control	WB: HeLa and C6 cell lysates treated with pervanadate. ICC/IF: HeLa cells treated with pervanadate. Flow Cyt: HeLa cells treated with pervanadate.
General notes	This protein is known to be similar in amino acid sequence to HCK (P08631), LCK (P06239), FYN (P06241), YES1 (P07947), and SRC (P12931). Therefore, cross-reactivity with these homologous proteins may be observed. We would be happy to provide immunogen alignment information upon request. 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 .

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 50% Glycerol (glycerin, glycerine), 0.05% BSA, 49% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP503Y
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab40660** in the following tested applications.

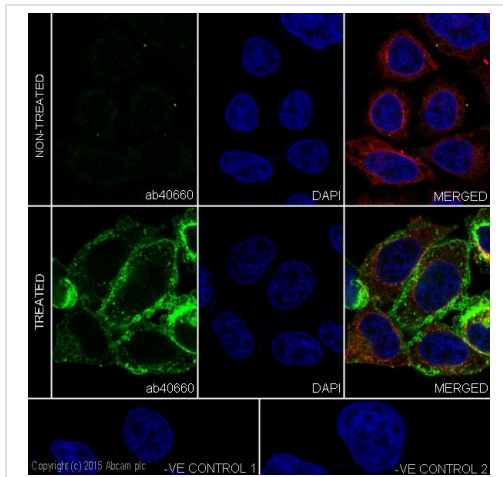
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★☆	1/1000 - 1/10000. Detects a band of approximately 56 kDa (predicted molecular weight: 61 kDa). Induction of cells with Pervanadate: Prepare 500mM stock Sodium Orthovanadate in PBS. To make 100mM (100x) Pervanadate Na3VO4 right before experiment, add 400ul 500mM Sodium Orthovanadate to 1560ul H2O and then 40ul H2O2, vortex and incubate at room RT for 5-10min. Cells are serum-starved overnight, and then treated with freshly made Pervanadate (100x, final concentration 1mM) for 20min at 37°C.
Flow Cyt		1/50. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.
ELISA		Use at an assay dependent concentration.

Target

Relevance	SRC Family Negative Regulatory Phosphorylation Site belongs to the protein kinase superfamily, Tyr protein kinase family, SRC subfamily. It contains one protein kinase domain, one SH2 domain and one SH3 domain. It may serve as part of a signaling pathway coupling the Fc receptor to the activation of the respiratory burst and may also contribute to neutrophil migration and may regulate the degranulation process of neutrophils. SRC Family Negative Regulatory Phosphorylation Site is expressed predominantly in cells of the myeloid and B lymphoid lineages. There are two named isoforms.
Cellular localization	Isoform 1: Membrane; Lipid anchor. Isoform 2: Membrane; Lipid anchor. Cytoplasm.

Images

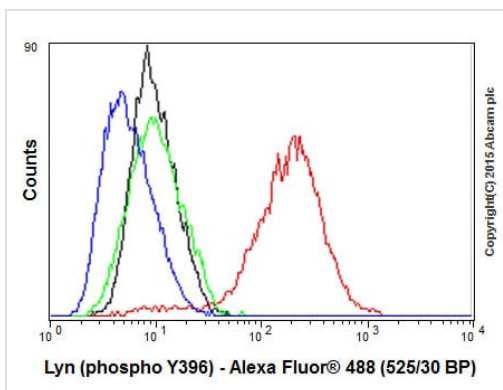


Immunocytochemistry/ Immunofluorescence - Anti-SRC Family (phospho Y418) antibody [EP503Y] (ab40660)

Immunocytochemistry/Immunofluorescence analysis of HeLa cells (untreated and treated with 1mM pervanadate for 15 minutes) labelling SRC Family (phospho Y418) with purified ab40660 at 1/250. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/1000) 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 (1/1000) were also used.

Control 1: primary antibody (1/250) and secondary antibody, ab150120, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/1000).

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



Flow Cytometry - Anti-SRC Family (phospho Y418) antibody [EP503Y] (ab40660)

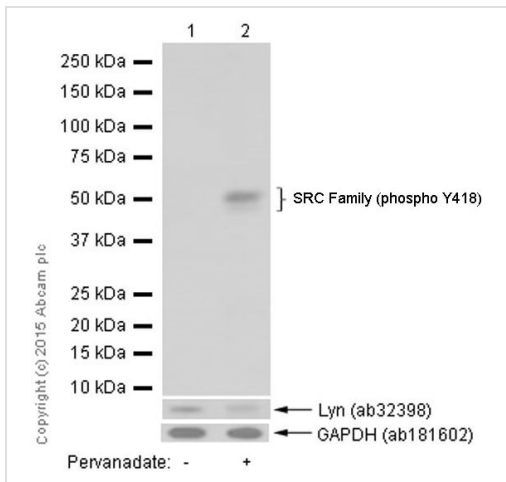
Flow Cytometry analysis of HeLa cells (treated with 1mM pervanadate for 30 minutes) labelling SRC Family (phospho Y418) with purified ab40660 at 1/80 (red). Cells were fixed with 4% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody.

Black - Isotype control, rabbit monoclonal IgG with pervanadate treated HeLa cells.

Blue - Unlabelled control, pervanadate treated HeLa cells without incubation with primary and secondary antibodies.

Red - Pervanadate treated HeLa cells with ab40660.

Green - Untreated HeLa cells labelled with ab40660.



Western blot - Anti-SRC Family (phospho Y418) antibody [EP503Y] (ab40660)

All lanes : Anti-SRC Family (phospho Y418) antibody [EP503Y] (ab40660) at 1/2000 dilution (purified)

Lane 1 : Untreated HeLa whole cell lysate

Lane 2 : Pervanadate treated HeLa whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

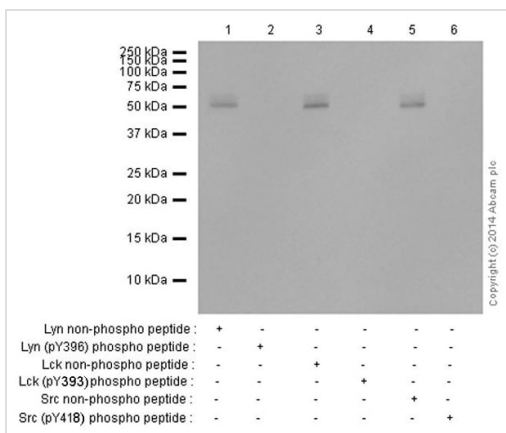
Predicted band size: 61 kDa

Observed band size: 56-59 kDa

[why is the actual band size different from the predicted?](#)

Exposure time: 3 seconds

Blocking and dilution buffer: 5% BSA/TBST.



Western blot - Anti-SRC Family (phospho Y418) antibody [EP503Y] (ab40660)

All lanes : Anti-SRC Family (phospho Y418) antibody [EP503Y] (ab40660) at 1/1000 dilution (purified)

Lane 1 : Pervanadate treated Jurkat cell lysate with Lyn non-phospho peptide

Lane 2 : Pervanadate treated Jurkat cell lysate with Lyn (pY396) phospho peptide

Lane 3 : Pervanadate treated Jurkat cell lysate with Lck non-phospho peptide

Lane 4 : Pervanadate treated Jurkat cell lysate with Lck (pY393) phospho peptide

Lane 5 : Pervanadate treated Jurkat cell lysate with Src non-phospho peptide

Lane 6 : Pervanadate treated Jurkat cell lysate with Src (pY418) phospho peptide

Secondary

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

Predicted band size: 61 kDa

Observed band size: 56-59 kDa [why is the actual band size](#)

different from the predicted?

Exposure time: 3 minutes

Blocking and dilution buffer: 5% NFDM/TBST.

All lanes : Anti-SRC Family (phospho Y418) antibody [EP503Y] (ab40660) at 1/1000 dilution (purified)

Lane 1 : Untreated C6 whole cell lysate

Lane 2 : Pervanadate treated C6 whole cell lysate

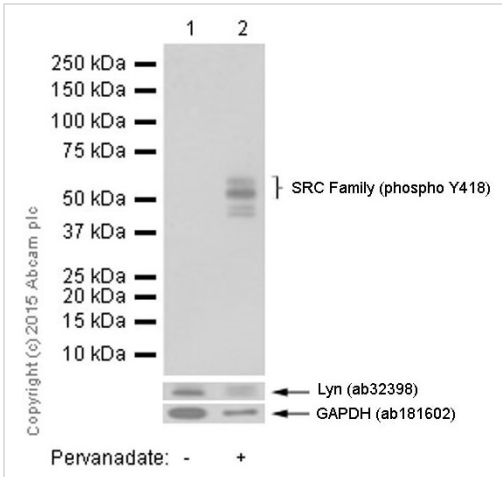
Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 61 kDa

Observed band size: 56-59 kDa [why is the actual band size different from the predicted?](#)

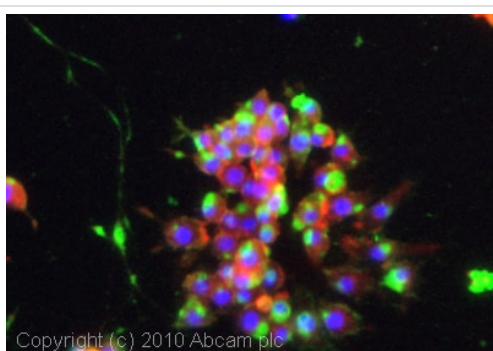


Western blot - Anti-SRC Family (phospho Y418) antibody [EP503Y] (ab40660)

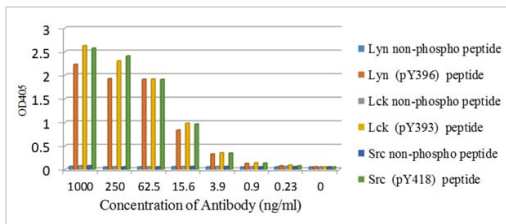
Exposure time: 1 second

Blocking and dilution buffer: 5% BSA/TBST.

ICC/IF image of unpurified ab40660 stained PC12 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab40660, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Immunocytochemistry/ Immunofluorescence - Anti-SRC Family (phospho Y418) antibody [EP503Y] (ab40660)



ELISA - Anti-SRC Family (phospho Y418) antibody
[EP503Y] (ab40660)

Direct ELISA antibody dose-response curve using unpurified ab40660 at 0-1000 ng/ml. Antigen concentration of 1000 ng/mL. An alkaline phosphatase-conjugated goat anti-rabbit IgG (H+L) (1/2500) was used as the secondary antibody.

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