abcam

Product datasheet

Anti-Src antibody [Clone 327] ab16885



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Overview

Product name Anti-Src antibody [Clone 327]

Description Mouse monoclonal [Clone 327] to Src

Host species Mouse

Specificity Reacts with both v-Src and c-Src proteins.

Tested applications Suitable for: Flow Cyt, WB

Unsuitable for: ICC/IF,IHC-Fr or IHC-P

Species reactivity Reacts with: Human

Immunogen Full length native protein (purified) corresponding to Src. Antibody generated by immunizing

BALB/c mice with full length native protein (purified) and then fusing with P3X63 Ag8.653

myeloma cells.

Epitope Epitope is within the SH3 domain of either v-Src or c-Src. We have no evidence for ab16885

cross-reacting with other src family kinases (e.g.fyn).

Positive control WB: MCF7, HeLa, A431 and NIH/3T3 cell lysates. Wild-type HAP1 cell lysate. Flow Cyt: SH-SY5Y

cells.

General notes May appear as a doublet due to phosphorylation. May be used to precipitate active Src which can

then be used in a kinase reaction.

This product was changed from ascites to tissue culture supernatant on 17 May 2019. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do

not hesitate to contact our scientific support team.

The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

Properties

Form Liquid

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Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer Preservative: 0.1% Sodium azide

Constituents: 0.2% Gelatin, 0.82% Sodium phosphate

Purity Tissue culture supernatant

Purification notes Purified from TCS.

Primary antibody notesMay appear as a doublet due to phosphorylation. May be used to precipitate active Src which can

then be used in a kinase reaction.

ClonalityMonoclonalClone numberClone 327

Myeloma P3x63-Ag8.653

Isotype IgG1

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab16885 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
Flow Cyt		Use at an assay dependent concentration. ab170190 - Mouse monoclonal lgG1, is suitable for use as an isotype control with this antibody.
WB	****(3)	Use at an assay dependent concentration. Predicted molecular weight: 59.7 kDa. ab16885 may appear as a doublet on WB due to phosphorylation. This antibody can be used to precipitate active src kinase.

Application notes Is unsuitable for ICC/IF,IHC-Fr or IHC-P.

Target

Function

Non-receptor protein tyrosine kinase that plays pivotal roles in numerous cellular processes such as proliferation, migration, and transformation. In concert with PTK2B, plays an important role in osteoclastic bone resorption. Both the formation of a SRC-PTK2B complex, and SRC kinase activity are necessary for this function. Once it is recruited to the activated integrins, by PTK2B, it phosphorylates CBL which in turn induces the activation and recruitment of phosphatidylinositol 3-kinase to the cell membrane in a signaling pathway that is critical for osteoclast function.

Promotes energy production in osteoclasts by activating mitochondrial cytochrome C oxidase.

Phosphorylates RUNX3 and COX2 on tyrosine residues, TNK2 on 'Tyr-284' and CBL on 'Tyr-731'.

Enhances DDX58/RIG-I-elicited antiviral signaling.

Sequence similarities

Belongs to the protein kinase superfamily. Tyr protein kinase family. SRC subfamily.

Contains 1 protein kinase domain.

Contains 1 SH2 domain.

Post-translational Dephosphorylated at Tyr-530 by PTPRJ (By similarity). Phosphorylated on Tyr-530 by c-Src

Contains 1 SH3 domain.

modifications

kinase (CSK). The phosphorylated form is termed pp60c-src. Dephosphorylated by PTPRJ at Tyr-419. Normally maintained in an inactive conformation with the SH2 domain engaged with Tyr-530, the SH3 domain engaged with the SH2-kinase linker, and Tyr-419 dephosphorylated. Dephosphorylation of Tyr-530 as a result of protein tyrosine phosphatase (PTP) action disrupts the intramolecular interaction between the SH2 domain and Tyr-530, Tyr-419 can then become autophosphorylated, resulting in SRC activation. Phosphorylation of Tyr-530 by CSK allows this interaction to reform, resulting in SRC inactivation.

Cellular localization

Cell membrane. Mitochondrion inner membrane.

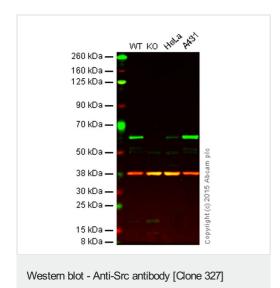
S-nitrosylation is important for activation of its kinase activity.

Form

This protein is known to be similar in amino acid sequence to HCK (P08631), LCK (P06239), FYN (P06241), YES1 (P07947), and LYN (P07948). Therefore, cross-reactivity with these homologous proteins may be observed. We would be happy to provide immunogen alignment information upon request.

Images

(ab16885)



Lane 1: Wild-type HAP1 cell lysate (20 µg)

Lane 2: Src knockout HAP1 cell lysate (20 µg)

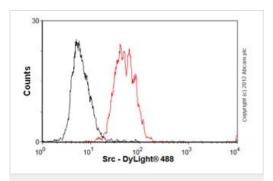
Lane 3: HeLa cell lysate (20 µg)

Lane 4: A431 cell lysate (20 µg)

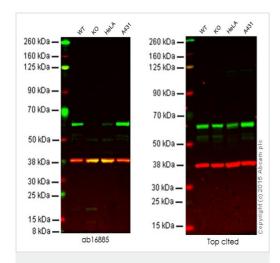
Lanes 1 - 4: Merged signal (red and green). Green - ab16885 observed at 60 kDa. Red - loading control, <u>ab8227</u>, observed at 42 kDa.

ab16885 was shown to specifically react with Src when Src knockout samples were used. Wild-type and Src knockout samples were subjected to SDS-PAGE. ab16885 and <u>ab8227</u> (loading control to beta Actin) were diluted 2.5µg/ml and 1/1000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (<u>ab216772</u>) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (<u>ab216777</u>) secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.

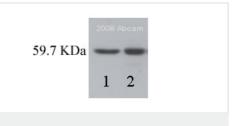
This image was generated using the ascites version of the product.



Flow Cytometry - Anti-Src antibody [Clone 327] (ab16885)



Western blot - Anti-Src antibody [Clone 327] (ab16885)



Western blot - Anti-Src antibody [Clone 327] (ab16885)

This image is courtesy of an anonymous Abreview

Overlay histogram showing SH-SY5Y cells stained with ab16885 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab16885, $1\mu g/1x10^6$ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, $2\mu g/1x10^6$ cells) used under the same conditions. Acquisition of >5,000 events was performed.

This image was generated using the ascites version of the product.

Lane 1: Wild-type HAP1 cell lysate (20 µg)

Lane 2: Src knockout HAP1 cell lysate (20 µg)

Lane 3: HeLa cell lysate (20 µg)

Lane 4: A431 cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab16885 observed at 60 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

This western blot image is a comparison between ab16885 and a competitor's top cited rabbit polyclonal antibody.

This image was generated using the ascites version of the product.

All lanes : Anti-Src antibody [Clone 327] (ab16885) at 2.5 μ g/ml

Lane 1 : Whole cell lysate prepared from human MCF-7 cells (Untreated)

Lane 2: Whole cell lysate prepared from human MCF-7 cells (Treated with 10uM PP2)

Secondary

All lanes : HRP conjugated Donkey polyclonal to mouse lg at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 59.7 kDa

Exposure time: 3 minutes

This image was generated using the ascites version of the product.

Anti-Src antibody [Clone 327] (ab16885) at 1/100 dilution + Lysate prepared from mouse NIH 3T3 cell line at 10 μg



HRP-conjugated sheep monoclonal to mouse IgG at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 59.7 kDa **Observed band size:** 60 kDa

Exposure time: 12 minutes

This image was generated using the ascites version of the product.

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-250 kDa

-100 kDa -75 kDa

-37kDa

-20 kDa

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Western blot - Anti-Src antibody [Clone 327]

This image is a courtesy of Anonymous Abreview

(ab16885)

We investigate all quality concerns to ensure our products perform to the highest standards

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