# abcam

## Product datasheet

# Anti-Src antibody ab47405

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Overview

Product name Anti-Src antibody

**Description** Rabbit polyclonal to Src

Host species Rabbit

Specificity This antibody detects endogenous levels of total Src protein. It will react with Src (pY529), Yes

(pY538), and Fyn (Y531).

Tested applications

Suitable for: ICC/IF, WB, IHC-P, ELISA

**Species reactivity** Reacts with: Mouse, Rat, Human, Pig

**Immunogen** Synthetic peptide corresponding to Human Src aa 487-536.

Database link: P12931

Positive control WB: HEK-293, HeLa cells; mouse primary astrocytes. IHC-P: Human breast carcinoma tissue.

ICC/IF: HEK-293 cells; pig retinal pigment epithelium cells.

**General notes**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

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: 7.40

Preservative: 0.02% Sodium azide

Constituents: PBS, 50% Glycerol, 0.87% Sodium chloride

PBS without Mg+2 and Ca+2

**Purity** Immunogen affinity purified

**Purification notes**The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-

1

specific immunogen.

**Clonality** Polyclonal

**Isotype** IgG

### **Applications**

### The Abpromise guarantee

Our Abpromise guarantee covers the use of ab47405 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	<b>★★★★ (3)</b>	Use a concentration of 1 - 5 μg/ml.
WB	<b>★★★★</b> (3)	1/500 - 1/1000. Detects a band of approximately 60 kDa (predicted molecular weight: 60 kDa).
IHC-P		Use at an assay dependent concentration.
ELISA		Use at an assay dependent concentration. Peptide ELISA only.

#### **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-l-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. Contains 1 SH3 domain.

# Post-translational modifications

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

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

### **Cellular localization**

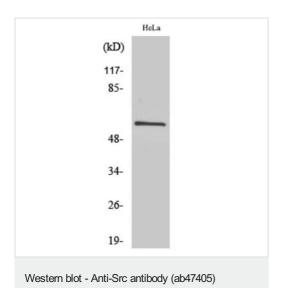
Cell membrane. Mitochondrion inner membrane.

### 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

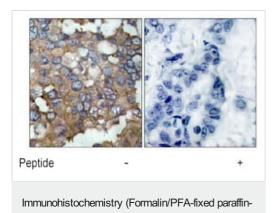
homologous proteins may be observed. We would be happy to provide immunogen alignment information upon request.

### **Images**



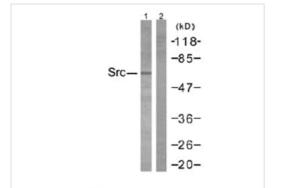
Anti-Src antibody (ab47405) at 1/2000 dilution + HeLa cells

Predicted band size: 60 kDa



embedded sections) - Anti-Src antibody (ab47405)

Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using Src antibody, in the presence and absence of blocking peptide.



Western blot - Anti-Src antibody (ab47405)

Peptide

All lanes: Anti-Src antibody (ab47405) at 1/500 dilution

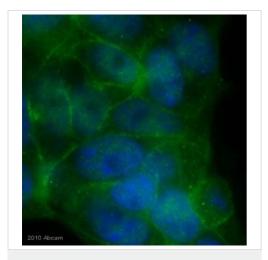
Lane 1: HEK-293 cell extracts

Lane 2: HEK-293 cell extracts with Blocking peptide

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

Western blot analysis of extracts from 293 cells using Src antibody in the presence and absence of blocking peptide. Western blot analysis of extracts from HEK-293 cells using Src antibody at 1/500

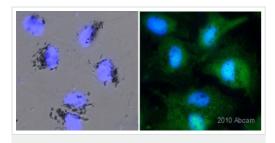
in the presence and absence of blocking peptide.



Immunocytochemistry/ Immunofluorescence - Anti-Src antibody (ab47405)

This image was kindly supplied by Dr Madimir Mlenkovic by Abreview

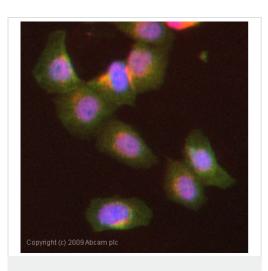
ab47405 staining Src in human HEK-293 cells by Immunocytochemistry/ Immunofluorescence. The cells were paraformaldehyde fixed, permeabilised in 0.5% Triton X-100 and then blocked using 5% serum for 20 minutes at 25°C. Samples were then incubated with primary antibody at 1/1000 for 16 hours at 4°C. The secondary antibody used was a goat anti-rabbit IgG conjugated to Alexa Fluor® 488 (green) used at a 1/5000 dilution. DAPI was used to stain the cell nuclei (blue).



Immunocytochemistry/ Immunofluorescence - Anti-Src antibody (ab47405)

This image is courtesy of an Abreview submitted by Dr. Madimir Mlenkovic.

ab47405 staining Src in Pig retinal pigment epithelium primary cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilzed with 0.5% Triton X-100 and blocked with 5% serum for 20 minutes at 25°C. Samples were incubated with primary antibody (1/1000 in 0.1% TX100, 1% goat serum, 1XPBS) for 16 hours at 4°C. An Alexa Fluor<sup>®</sup>488-conjugated goat anti-rabbit IgG polyclonal (1/5000) was used as the secondary antibody. Nuclei were counterstained with DAPI.



Immunocytochemistry/ Immunofluorescence - Anti-Src antibody (ab47405)

ICC/IF image of ab47405 stained MCF7 cells (ab3871). The cells were 100% methanol fixed (5 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 (ab47405, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit lgG (H+L) (ab150077) 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.



Anti-Src antibody (ab47405) at 1/500 dilution + Recombinant human Src protein (ab79635) at 0.001 µg

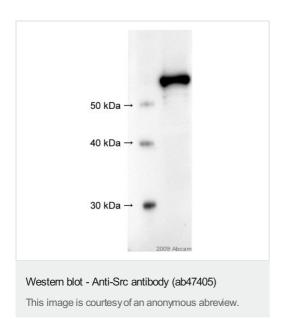
### **Secondary**

Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 60 kDa



Exposure time: 10 seconds

Anti-Src antibody (ab47405) at 1/1000 dilution + whole cell lysate prepared from mouse primary astrocytes at 15  $\mu$ g

### **Secondary**

Goat abti-rabbit IgG conjugated to HRP at 1/5000 dilution

Performed under reducing conditions.

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

Exposure time: 5 seconds

Primary antibody incubated for 1 hour at 25°C.

Blocked with 3& BSA for 1 hour at 25°C.

Gel run under denaturing conditions.

Detection method: ImmunoStar.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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