abcam

Product datasheet

Anti-Src antibody [EGTR103] - BSA and Azide free ab222221

KO VALIDATED Recombinant RabMAb

9 Images

Overview

Product name	Anti-Src antibody [EGTR103] - BSA and Azide free
Description	Rabbit monoclonal [EGTR103] to Src - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Rat and Mouse colon tissues. Human liver tissue. WB: HAP1, A431, U87-MG, and SH-5YSY cell lysates. Mouse and rat brain lysate. Mouse hippocampus lysate
General notes	ab222221 is the carrier-free version of **ab133283**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EGTR103
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab222221 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		Use at an assay dependent concentration. Predicted molecular weight: 60 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.

Target

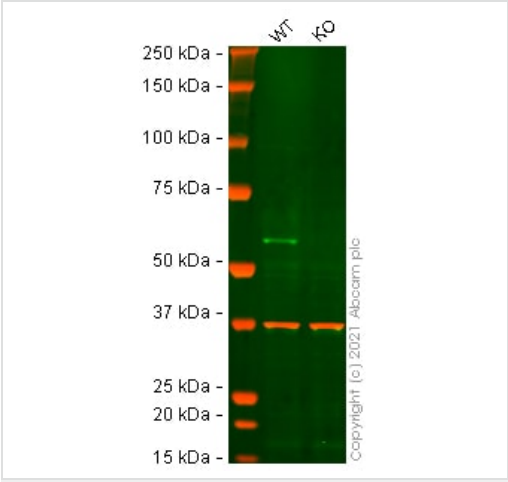
Function	Non-receptor protein tyrosine kinase that plays pivotal roles in numerous cellular processes such as proliferation, migration, and transformation. In concert with PTK2B, plays an important role in osteoclastic bone resorption. Both the formation of a SRC-PTK2B complex, and SRC kinase activity 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. 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



Western blot - Anti-Src antibody [EGTR103] - BSA and Azide free (ab222221)

All lanes : Anti-Src antibody [EGTR103] (**ab133283**) at 1/1000 dilution

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : SRC knockout HAP1 cell lysate

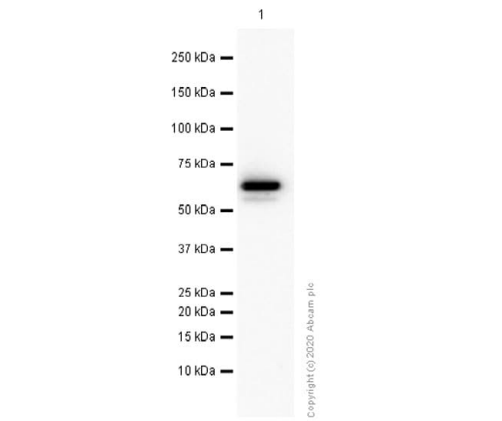
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 60 kDa
Observed band size: 65 kDa

False colour image of Western blot: Anti-Src antibody [EGTR103] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab133283** was shown to bind specifically to Src. A band was observed at 65 kDa in wild-type HAP1 cell lysates with no signal observed at this size in SRC knockout cell line HAP1. To generate this image, wild-type and SRC knockout HAP1 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW)

preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



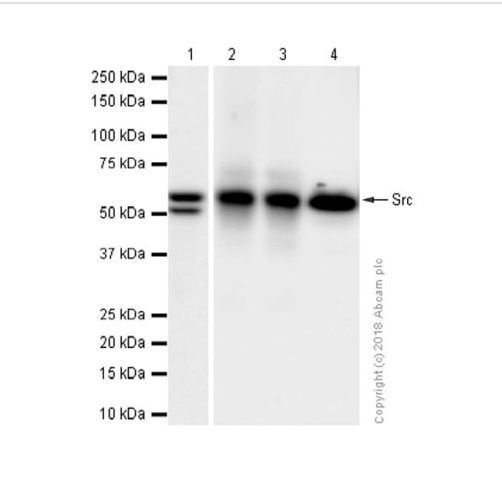
Western blot - Anti-Src antibody [EGTR103] - BSA and Azide free (ab222221)

Anti-Src antibody [EGTR103] (**ab133283**) at 1/2000 dilution + U-87 MG (Human glioblastoma-astrocytoma epithelial cell) whole cell lysate at 15 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

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

This data was developed using **ab133283**, the same antibody clone in a different buffer formulation.



Western blot - Anti-Src antibody [EGTR103] - BSA and Azide free (ab222221)

All lanes : Anti-Src antibody [EGTR103] (**ab133283**) at 1/1000 dilution (Purified)

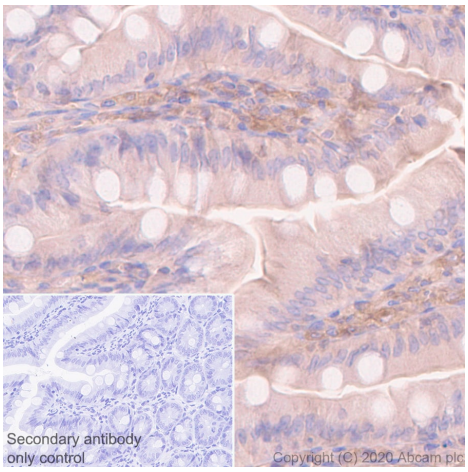
Lane 1 : SH-SY5Y (Human neuroblastoma epithelial cell) whole cell lysate
Lane 2 : Mouse brain lysate
Lane 3 : Rat brain lysate
Lane 4 : Mouse hippocampus lysate

Lysates/proteins at 20 µg per lane.

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

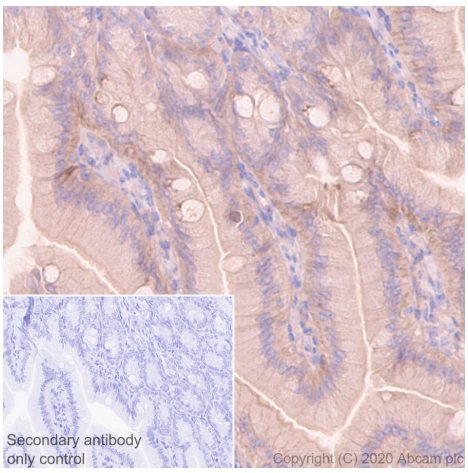
Predicted band size: 60 kDa
Observed band size: 60 kDa

This data was developed using **ab133283**, the same antibody clone in a different buffer formulation.



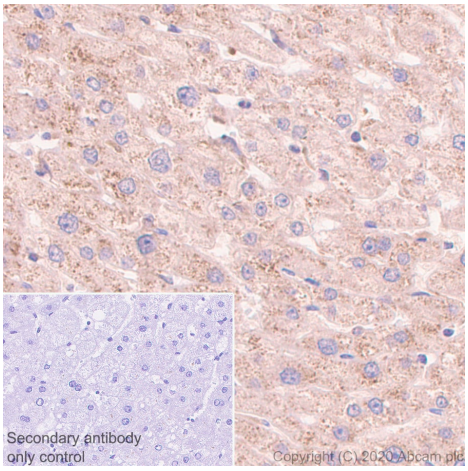
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Src antibody [EGTR103] - BSA and Azide free (ab222221)

This data was developed using **ab133283**, the same antibody clone in a different buffer formulation. Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat colon tissue sections labeling Src with purified **ab133283** at 1/50 dilution (6.98 µg/mL). Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



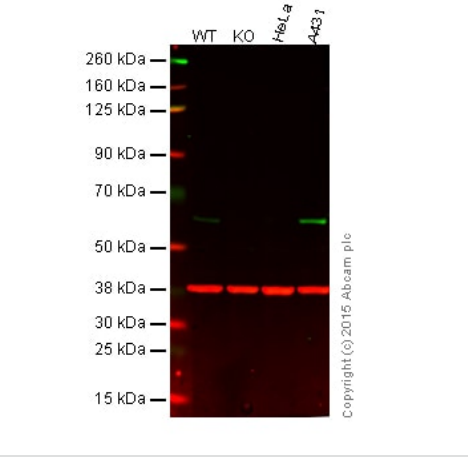
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Src antibody [EGTR103] - BSA and Azide free (ab222221)

This data was developed using **ab133283**, the same antibody clone in a different buffer formulation. Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse colon tissue sections labeling Src with purified **ab133283** at 1/50 dilution (6.98 µg/mL). Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Src antibody [EGTR103] - BSA and Azide free (ab222221)

This data was developed using **ab133283**, the same antibody clone in a different buffer formulation.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue sections labeling Src with purified **ab133283** at 1/50 dilution (6.98 µg/mL). Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-Src antibody [EGTR103] - BSA and Azide free (ab222221)

This data was developed using **ab133283**, the same antibody clone in a different buffer formulation.

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

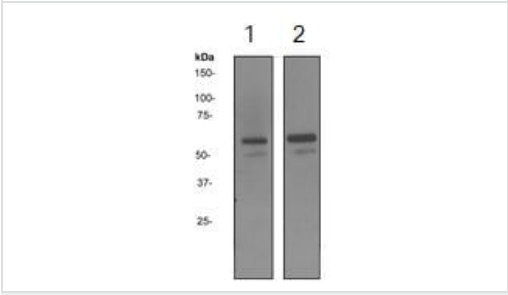
Lane 2 Src knockout HAP1 cell lysate (20 µg)

Lane 3 HeLa cell lysate (20 µg)

Lane 4 A431 knockout HAP1 cell lysate (20 µg)

Lanes 1 - 4 Merged signal (red and green). Green - **ab133283** observed at 60 kDa. Red - loading control, **ab8226**, observed at 42 kDa.

Unpurified **ab133283** was shown to specifically react with Src when Src knockout samples were used. Wild-type and Src knockout samples were subjected to SDS-PAGE. **ab133283** and **ab8226** (loading control to beta actin) were both diluted 1/1000 and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW)preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-Src antibody [EGTR103] - BSA and Azide free (ab222221)

All lanes : Anti-Src antibody [EGTR103] (**ab133283**) at 1/1000 dilution (Unpurified)

Lane 1 : U87-MG cell lysate

Lane 2 : SH-5YSY cell lysate

Secondary

All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 60 kDa

This data was developed using **ab133283**, the same antibody clone in a different buffer formulation.

Anti-Src antibody [EGTR103] - BSA and Azide free (ab222221)

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