Anti-Src Antibody [JF0947]

ET1702-03

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 60 kDa
Clone number:	JF0947

Description: The major translational products of the Src gene family are membrane-associated tyrosine protein kinases that lack transmembrane and external amino acid sequences. By virtue of their common structural motifs, the Src family is composed of nine members in vertebrates, including c-Src, c-Yes, Fgr, Yrk, Fyn, Lyn, Hck, Lck and Blk. Src family kinases, which contain an amino-terminal cell membrane anchor followed by SH3 and SH2 domains, transduce signals that are involved in the control of a variety of cellular processes, including proliferation, differentiation, motility and adhesion. Src family members are normally maintained in an inactive state and can be activated transiently during cellular events such as mitosis. Different subcellular locations of Src family kinases may be important for the regulation of specific cellular processes, such as mitogenesis, cytoskeletal organization and membrane trafficking. c-Src (also designated pp60Src, Src p60 and proto-oncogene tyrosine protein kinase Src) is expressed in a broad range of tissue and cell types, although the highest levels of c-Src are detected in neuronal tissues and platelets. c-Src may play a role in events associated with both neuronal differentiation and maintenance of mature neuronal cell functions.

Immunogen: Synthetic peptide within human Src aa 20-60.

Positive control: SK-OV-3 cell lysate, A549 cell lysate, NIH/3T3 cell lysate, 4T1 cell lysate, PC-12 cell lysate, C6 cell lysate, COS-1 cell lysate, SK-OV-3, NIH/3T3, PC-12, human kidney tissue, mouse kidney tissue, rat kidney tissue, SK-OV-3 Y.

Subcellular location: Cell membrane, Mitochondrion inner membrane, Nucleus, Cytoplasm.

Database links: SwissProt: P12931 Human | P05480 Mouse | Q9WUD9 Rat

Recommended Dilutions:

WB	1:1,000-1:5,000
IF-Cell	1:100-1:500
IF-Tissue	1:100-1:500
IHC-P	1:200-1:1,000
IP	1/2,000
FC	1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

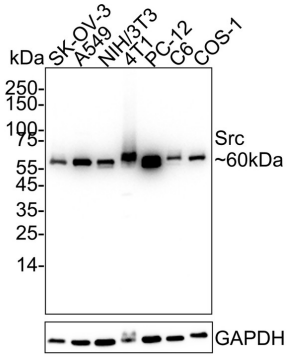

Fig1: Western blot analysis of Src on different lysates with Rabbit anti-Src antibody (ET1702-03) at 1/1,000 dilution.

Lane 1: SK-OV-3 cell lysate, 20 µg/Lane
Lane 2: A549 cell lysate, 20 µg/Lane
Lane 3: NIH/3T3 cell lysate, 20 µg/Lane
Lane 4: 4T1 cell lysate, 20 µg/Lane
Lane 5: PC-12 cell lysate, 20 µg/Lane
Lane 6: C6 cell lysate, 20 µg/Lane
Lane 7: COS-1 cell lysate, 20 µg/Lane

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 16 seconds;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1702-03) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

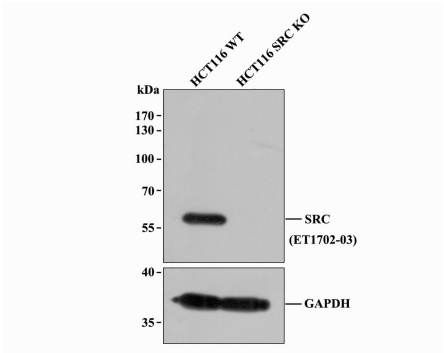

Fig2: All lanes: Western blot analysis of Src with anti-Src antibody [JF0947] (ET1702-03) at 1:1,000 dilution.

Lane 1: Wild-type HCT116 whole cell lysate (20 µg).
Lane 2: Src knockout HCT116 whole cell lysate (20 µg).

ET1702-03 was shown to specifically react with Src in wild-type HCT116 cells. No band was observed when Src knockout sample was tested. Wild-type and Src knockout samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1702-03, 1/1,000) and Loading control antibody (Rabbit anti-GAPDH , ET1601-4, 1/10,000) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG-HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

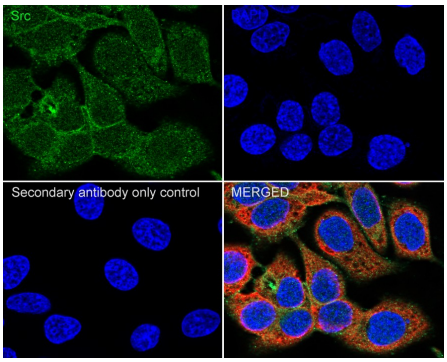

Fig3: Immunocytochemistry analysis of SK-OV-3 cells labeling Src with Rabbit anti-Src antibody (ET1702-03) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Src antibody (ET1702-03) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

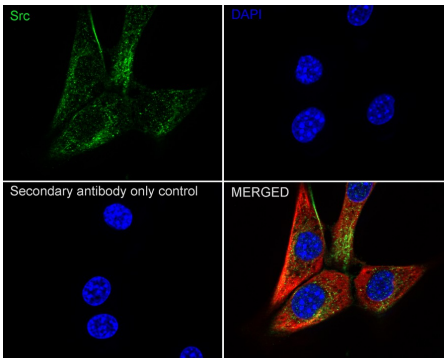

Fig4: Immunocytochemistry analysis of NIH/3T3 cells labeling Src with Rabbit anti-Src antibody (ET1702-03) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Src antibody (ET1702-03) at 1/50 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

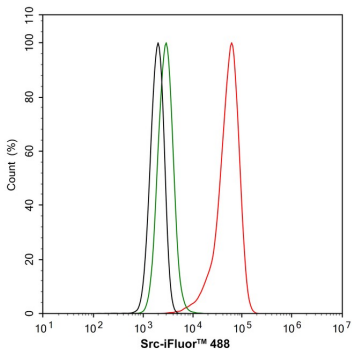

Fig5: Flow cytometric analysis of SK-OV-3 cells labeling Src.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1702-03, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

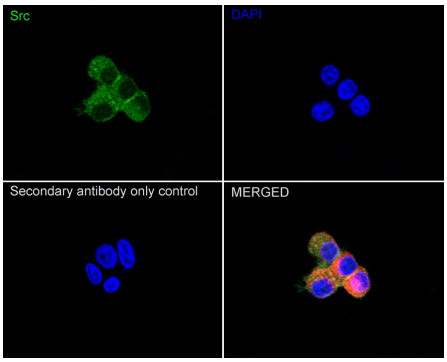

Fig6: Immunocytochemistry analysis of PC-12 cells labeling Src with Rabbit anti-Src antibody (ET1702-03) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Src antibody (ET1702-03) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

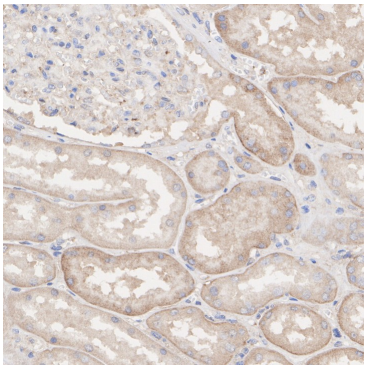

Fig7: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Src antibody (ET1702-03) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1702-03) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

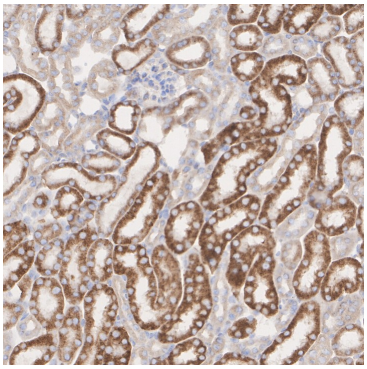

Fig8: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Src antibody (ET1702-03) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1702-03) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

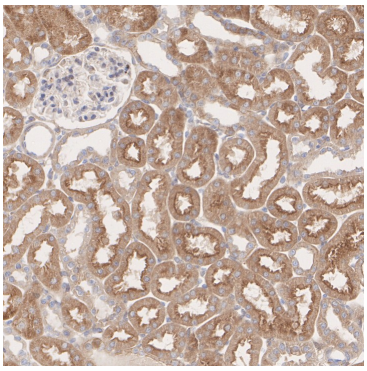

Fig9: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-Src antibody (ET1702-03) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1702-03) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

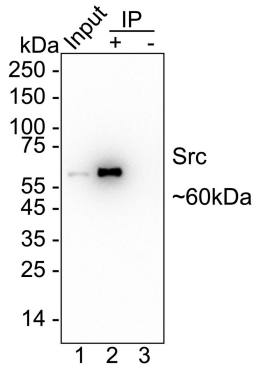

Fig10: Src was immunoprecipitated in 0.2mg A549 cell lysate with (ET1702-03) at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using (ET1702-03) at 1/2,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: A549 cell lysate (input)
Lane 2: (ET1702-03) IP in A549 cell lysate
Lane 3: Rabbit IgG instead of (ET1702-03) in A549 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 6s

Background References

1. Kumar R et al. Identification and characterization of the role of c-terminal Src kinase in dengue virus replication. Sci Rep 6:30490 (2016).
2. Almeida MT et al. Src-dependent tyrosine phosphorylation of non-muscle myosin heavy chain-IIA restricts Listeria monocytogenes cellular infection. J Biol Chem 290:8383-95 (2015).