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Polyclonal Antibody to c-src (31-49) - Aff - Purified

Catalog No.: BP7215

Quantity: 0.1 ml

Background: Src (also known as pp60src) is a non-receptor tyrosine kinase involved in signal transduction in many biological systems and implicated in the development of human tumors. This kinase is expressed in different tissues of the body with the highest protein levels detected in neurons and platelets. Src can modulate the signal transduction pathways activated by several growth factors (e.g., PDGF, M-CSF and G-CSF) and integrins. Src also regulates the activity of several ion channels including the N-methyl-D-aspartate (NMDA) receptor. In addition, Src is thought to play a role in physiological/pathophysiological processes in the central nervous system. This antibody is useful to determine total levels of Src protein.

Host: Rabbit

Immunogen: Chemically synthesized peptide derived from the amino acid region 31-49 of human Src protein.
Remarks: The sequence is conserved in mouse and rat.

Format: **State:** Liquid Ig fraction
Purification: Epitope-specific affinity chromatography
Buffer System: Dulbecco's phosphate buffered saline (without Mg2+ and Ca2+), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier, containing 0.05 % sodium azide

Applications: Western blot (0.25-1.0 µg/ml).
Positive Controls Used: Primary chicken embryo fibroblasts (CEF) expressing human wild-type Src protein.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody detects Src.
Species: Human, Mouse, Rat.
Other species not tested.

Add. Information: 69 % homologous with chicken.

Storage: Store at 2 - 8 °C up to one week or (in aliquots) at -20 °C for longer. Avoid repeated freezing and thawing.
Centrifuge vial before opening.
Shelf life: one year from despatch.

General Readings: Schliess, F., et al. (2004) Involvement of integrins and src in insulin signaling toward autophagic proteolysis in rat liver. J. Biol. Chem. 279(20):21294-21301.
Sato, K., et. al. (2003) Src-dependent phosphorylation of the EGF receptor Tyr-845 mediates Stat-p21waf1 pathway in A431 cells. Genes Cells. 8(12):995-1003.

Moro, L., et al. (2002) Integrin-induced epidermal growth factor (EGF) receptor activation requires c-Src and p130Cas and leads to phosphorylation of specific EGF receptor tyrosines. |J. Biol. Chem. 277(11):9405-9414.
Roy, S., et al. (2002) FAK regulates tyrosine phosphorylation of CAS, paxillin, and PYK2 in cells expressing v-Src, but is not a critical determinant of v-Src transformation. J. Cell. Biochem. 84(2):377-388.
Simeonova, P.P., et al. (2002) c-Src-dependent activation of the epidermal growth factor receptor and mitogen-activated protein kinase pathway by arsenic. Role in carcinogenesis. J. Biol. Chem. 277(4):2945-2950.
Cheng, A., et al. (2001) Attenuation of adhesion-dependent signaling and cell spreading in transformed fibroblasts lacking protein tyrosine phosphatase-1B. J. Biol. Chem. 276(28):25848-25855.
Dorey, K., et al. (2001) Phosphorylation and structure-based functional studies reveal a positive and a negative role for the activation loop of the c-Abl tyrosine kinase. Oncogene 20(56):8075-8084.
Nakamura, K., et al. (2001) Different modes and qualities of tyrosine phosphorylation of Fak and Pyk2 during epithelial-mesenchymal transdifferentiation and cell migration: analysis of specific phosphorylation events using site-directed antibodies. Oncogene 20(21):2626-2635.
Nanki, T., et al. (2001) Chemokines regulate IL-6 and IL-8 production by fibroblast-like synoviocytes from patients with rheumatoid arthritis. J. Immunol. 167(9):5381-5385.
Ruest, P.J., et al. (2001) Mechanisms of CAS substrate domain tyrosine phosphorylation by FAK and Src. Mol. Cell. Biol. 21(22):7641-7652.
Schaller, M.D. (2001) Paxillin: a focal adhesion-associated adaptor protein. Oncogene 20(44):6459-6472.

Protocols:

Western Blotting Procedure

1. Lyse approximately 10e7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifugedat 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH2O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.

11. Block the membrane with blocking buffer (formulation provided below) for one hour at room temperature or overnight at 4°C.
12. Incubate the blocked blot with primary antibody at a concentration of 0.1-1.0 µg/mL in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween 20 overnight at 4°C or for 2 hours at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat F(ab)2 anti-rabbit IgG alkaline phosphatase conjugate or goat F(ab)2 anti-rabbit IgG horseradish peroxidase conjugate in conjunction with your chemiluminescence reagents and instrumentation.

Cell Lysis Buffer Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM Na4P2O7
2 mM Na3VO4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO)
or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma Cat. # P2714 may be used)

Transfer Buffer Formulation:
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add 1 mL 10% SDS.
Cool to 4°C prior to use.

Tris Buffered Saline Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer Formulation:
100 mL Tris buffered saline
5 gm BSA
0.1 mL Tween 20

Pictures:

Western Blot Analysis Extracts of CEFs untransfected (-) or transfected (+) with wild-type human Src were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to nitrocellulose. The membrane was blocked with a 5% BSA-TBST buffer overnight at 4oC, then incubated with 0.5 µg/mL Src pan antibody for two hours at room temperature in a 3% BSA-TBST buffer. After washing, the membrane was incubated with goat F(ab')2 anti-rabbit IgG alkaline phosphatase and signals were detected using the Tropix WesternStar™ method. The data show the total level of Src present in each condition