Rabbit (polyclonal) Anti-Src Pan, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number:	44-655G
Lot Number:	See product label
Volume:	200 µL
Form of Antibody:	Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol, with 1.0 mg/mL BSA (IgG, protease free) as a carrier.
Preservative:	0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)
Purification:	Purified from rabbit serum by epitope-specific chromatography.
Immunogen:	The antibody was produced using a synthetic peptide derived from the amino acid region 6-19 of human Src. The sequence is conserved in mouse and rat.
Target Summary:	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.
Reactivity:	Human and mouse Src. Rat (100% homologous) and chicken Src (93% homologous) have not been tested, but are expected to react.
Applications:	The antibody has been used for Western blotting applications.
Suggested Working Dilutions:	For Western blotting applications, we recommend using the antibody at a 1:1000 dilution. The optimal antibody concentration should be determined empirically for each specific application.
Storage:	Store at –20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at –20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.
Expiration Date:	Expires one year from date of receipt when stored as instructed.
Positive Control Used:	CEF cell extracts +/- Src; human platelets
Related Products:	**Sample Packs:** FAK sample pack, Cat. # 44-631G Pyk2 sample pack, Cat. # 44-638G **Extracts:** CEF cell extracts +/- Src, Cat. # 55-120

This product is for research use only. Not for use in diagnostic procedures.

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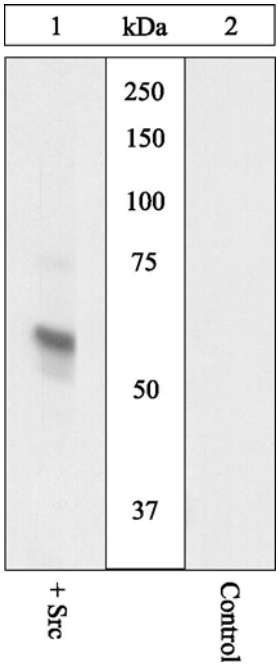
PI44655G (Rev 11/08) DCC-08-1089

References:

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.



Western Blot

Extracts prepared from CEF cells transfected with Src (1) or left untransfected (2) were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF. Membranes were blocked with a 5% BSA-TBST buffer for two hours at room temperature and incubated with a 1:1000 dilution of Src pan antibody for two hours at room temperature in a 1% BSA-TBST buffer. After washing, membranes were incubated with goat $F(ab')_2$ anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and bands were detected using the Pierce SuperSignal™ method.

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Western Blotting Procedure

1. Lyse approximately 10^7 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. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Cat. # FNN0011). 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 ultracentrifuged at 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 ddH_2O 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 1:1000 dilution in Tris buffered saline supplemented with 1% BSA and 0.1% Tween 20 overnight at 4°C or for one hour 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 (Cat. # ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (Cat. # ALI4404) 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 $Na_4P_2O_7$
2 mM Na_3VO_4
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

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