Rabbit (polyclonal) Anti-Src [pY^418] Phosphorylation Site Specific Antibody Alexa Fluor® 488 Conjugate

PRODUCT ANALYSIS SHEET

Catalog Number: 44660A1 (50 Tests)
Lot Number: See product label
Volume: 100 μL
Suggested Dilution: The optimal dilution should be determined empirically for each cell type and stimulation protocol. The recommended starting dilution for most experimental systems is 1:50.
Formulation: Alexa Fluor® 488-conjugated purified immunoglobulin in Dulbecco’s phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), with 0.2% BSA and 0.05% sodium azide. (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)
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. Tyrosine 418 is located in the catalytic SH2 domain and is one of the autophosphorylation sites. Full catalytic activity of Src requires phosphorylation of tyrosine 418. This activity is negatively regulated when tyrosine 529 of Src is phosphorylated by Csk. This conformation holds Src in the inactive form through an intramolecular interaction between the SH2 domain and the carboxyl terminus.
Reactivity: Human, dog, mouse, and chicken Src. This antibody also reacts with Fyn and Yes.

Immunofluorescence Staining:
MDCK cells were left untreated (A), stimulated with H_{2}O_{2} (1 mM for 10 minutes) (B) or pretreated with PP2 prior to H_{2}O_{2} stimulation (C). Cells were then fixed prior to immunostaining with the anti-Src [pY^418] Alexa Fluor® 488 conjugate. The data show that the fluorescence signal is induced in H_{2}O_{2}-treated cells, and blocked in cells pretreated with Src kinase inhibitor (PP2), indicating that the signal is Src phospho-specific.

Please visit our website (Invitrogen.com) to view the images in full color.

Production: This antibody was produced against a chemically synthesized phosphopeptide derived from the region of Src that contains tyrosine 418. The antibody was affinity purified by sequential epitope-specific chromatography, then conjugated to Alexa Fluor® 488 under optimal conditions.

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

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(Rev 03/09) DCC-09-0300
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Storage: Store at 2-8°C for up to one month. For long term storage, apportion the antibody into working aliquots and store at -20°C. Avoid repeated freeze-thaw cycles to prevent denaturing the antibody. Protect from light.

Expiration Date: Expires one year from date of receipt when stored as instructed.

Related Products: Antibodies:
- Paxillin [pY118], Cat. # 44722G
- Paxillin [pY31], Cat. # 44720G
- FAK [pY397], Cat. # 44624G
- FAK [pY576], Cat. # 44652G
- Alexa 488 Pyk2 [pY402], Cat. # 44618A1
- Alexa 488 Paxillin [pY118], Cat. # 44722A1
- Alexa 488 ERK1/2 [pTpY185/187], Cat. # 44680A1
- Alexa 488 PRAS40 [pT246], Cat. # 441100A1

References:
- Inhibition of focal adhesion kinase expression or activity disrupts epidermal growth factor-stimulated signaling promoting the migration of invasive human carcinoma cells. Cancer Res. 61(19):7079-7090.

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Immunofluorescence Staining of Adherent Cells:
Invitrogen’s phosphorylation site specific antibodies have been used successfully in many antibody-based techniques, including Western blot analyses and sandwich immunoassays (i.e., phosphoELISA™ and Luminex™ assays). When used in immunohistochemistry, these antibodies not only detect phosphorylation events, but also provide valuable information about the subcellular localization of phosphorylated proteins. Invitrogen’s Alexa Fluor® 488-conjugated phosphorylation site specific antibodies have been developed specifically to provide a facile method for detecting protein phosphorylation and localization by immunohistochemistry.

Procedure:
1. Plate adherent cells onto glass cover slips.
2. Culture the cells for 16 hours in appropriate medium. It is important to note that serum starvation may be necessary in some stimulation procedures.
3. Stimulate the cells as desired.
4. Remove the medium from the cells by decanting.
5. Fix the cells by pipetting 200 μL 95% ice cold methanol onto the slides. (Fixatives composed of equal volumes of acetone and methanol, or 4% paraformaldehyde, have also been used successfully.)
6. Incubate for 10 minutes at -20°C.
7. Remove the fixative solution by decanting.
8. Pipette 200 μL Blocking Buffer onto the slides. (Blocking Buffer Formulation: 3% BSA/TBST/0.1% Triton X-100, supplemented with protease inhibitor cocktail [Sigma Cat. # P8340] and phosphatase inhibitor cocktail I and II [Sigma Cat. # P2850 and P5726]).
9. Incubate for 30 minutes at room temperature.
10. Remove the Blocking Buffer by decanting.
11. Pipette 100-200 μL Alexa Fluor® 488 conjugated phosphorylation site specific antibody, diluted in Blocking Buffer, onto the cover slip.
12. Incubate for two hours at room temperature or overnight at 2-8°C.
13. Remove the antibody solution by decanting.
14. Wash the cells twice with phosphate buffered saline, pH 7.2, 10 minutes each.
15. Add one drop of Vectashield solution (Vector Lab, H-1500) to prevent photobleaching the fluorescent signal. Vectashield solution contains DAPI to counterstain the nucleus. Mount the cover slip on a microscopic slide.
16. Examine the slides with an immunofluorescence microscope (e.g., Zeiss Axioplan 2). We suggest using the 100x oil immersion lens.