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ProductInformation

Anti-phospho-Src (pTyr^{529})
Developed in Rabbit, Affinity Purified Antibody

Product Number **S 2065**

Product Description

Anti-phospho-Src (pTyr^{529}) was developed in rabbit using a synthetic phosphopeptide derived from the region of human Src that contains tyrosine 529 (tyrosine 530 including the initiating methionine). as immunogen. The sequence is conserved in mouse, rat, chicken and frog. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed using (1) a non-phosphorylated peptide to remove any reactivity toward non-phosphorylated Src, and (2) a generic tyrosine phosphorylated peptide to remove any reactivity toward phosphotyrosine that is not sequence specific.

Anti-phospho-Src (pTyr^{529}) recognizes human, mouse, rat and chicken Src (60 kDa). Fyn and Yes (92% homologous) were not tested. It has been used in immunoblotting and immunohistochemistry applications.

c-Src was one of the first cellular proteins demonstrated to have tyrosine kinase activity. As such, Src is a prototype for understanding signal transduction events involving tyrosine phosphorylation.[1]

The non-receptor c-Src family kinases include Blk, c-Fgr, Fyn, Hck, Lck, Lyn, c-Scr, ZAP-70, c-Yes and Yrk. Each encodes a cytoplasmic protein-tyrosine kinase (PTK) believed to be involved in signal transduction.[2] The c-Src PTKs contain three domains (SH1, SH2 and SH3) that are found in many other signaling proteins. The SH1 domain has PTK activity. SH2 and SH3 domains mediate protein-protein interactions by binding to phosphotyrosine-containing and proline-rich motifs, respectively.[3] c-Src PTKs function in a broad range of biological situations, including T-lymphocyte maturation and activation, keratinocyte differentiation, bone maintenance, and learning.[4]

Three tyrosine phosphorylation sites on the molecule control the kinase activities of Src. Activity is downregulated *in vivo* by autophosphorylation of Tyr^{527} near the carboxyl terminus of chicken Src (Tyr^{529} on human c-Src, Tyr^{534} on mouse c-Src, and Tyr^{525} on frog c-Src). Phosphorylation at Tyr^{527} is primarily intramolecular and induces the kinase to form an inactive conformation.[6,7] Receptor protein tyrosine phosphatase-α (RPTPα) was recently identified as a physiological upstream activator of Src-family kinases.

Autophosphorylation of chicken c-Src also occurs on Tyr^{416} (Tyr^{414} on frog c-Src, Tyr^{418} on human c-Src, and Tyr^{423} on mouse c-Src). and is readily demonstrated *in vitro*. Phosphorylation of Tyr^{416} is mostly intermolecular since its rate is concentration-dependent. This site is located in the catalytic domain of human Src, [6] and phosphorylation of this site is required for full catalytic activity

PDGF receptor phosphorylation of chicken c-Src on Tyr^{213} (Tyr^{215} on human c-Src, Tyr^{211} on frog c-Src, and Tyr^{220} on mouse c-Src) specifically blocks binding of its SH2 domain to a phosphopeptide corresponding to the C-terminal regulatory sequence and results in a 50-fold activation of Src.[7] Phosphorylation at this site overcomes the down regulation induced by phosphorylation at Tyr^{527}.

Reagent

The antibody is supplied as 100 µL of solution in Dulbecco's phosphate-buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, containing 50% glycerol, 1.0 mg/ml BSA (IgG and protease-free) and 0.05% sodium azide.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

Store at –20 °C. Centrifuge briefly before opening to settle vial contents. Then, store in working aliquots. The antibody is stable at 2-8 °C for up to one week. Working dilutions should be discarded if not used within 12 hours.

Product Profile
A recommended working dilution of 1:1000 is determined by immunoblotting. .

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration.

Peptide Competition

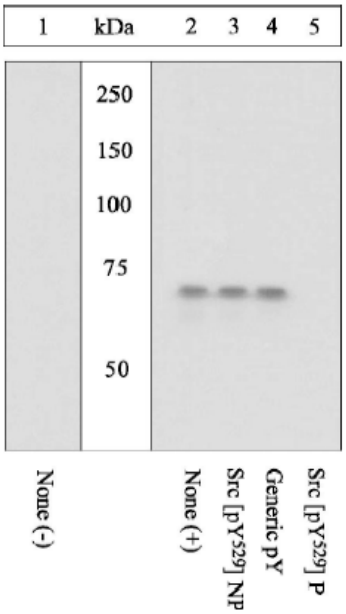

Figure 1

1. Extracts and membranes were prepared using CEF cells transfected with wild type (WT) Src and PVDF membranes.
2. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C.
3. The Src [pTyr^{529}] antibody was preincubated with different peptides as follows:
   Lane 1,2 no peptide
   Lane 3 non-phosphorylated peptide corresponding to the immunogen
   Lane 4 a generic phosphotyrosine containing peptide
   Lane 5 immunogen
4. The preincubated peptide-antibody was added to the membranes and incubated 1 hour at room temperature or overnight at 2-8 °C.
5. Membranes were washed four times
6. After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG-HRP and signals were detected.

The data in Figure 1 show that only the peptide corresponding to Src [pTyr^{529}] blocks the antibody signal, thereby demonstrating the specificity of the antibody.

References

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AH,PHC 01/05-1