

Product Information

Anti-phospho-Src (pTyr⁴¹⁸)

Developed in Rabbit, Affinity Purified Antibody

Product Number **S 1940**

Product Description

Anti-phospho-Src (pTyr⁴¹⁸) was developed in rabbit using as immunogen a synthetic phosphopeptide derived from the region of Src that contains tyrosine 418, based on the sequence of human Src. The sequence is conserved in human, mouse, chicken and frog. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preabsorbed using (1) a non-phosphorylated peptide to remove any reactivity toward the non-phosphorylated Src enzyme, and (2) a generic tyrosine phosphorylated peptide to remove any reactivity toward phosphotyrosine that is not sequence specific.

Anti-phospho-Src (pTyr⁴¹⁸) recognizes human, mouse, rat and chicken Src (60 kDa). It has been used in immunoblotting applications.

c-Src is 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-Src, ZAP-70, cYes 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

The kinase activities of Src are controlled by three tyrosine phosphorylation sites on the molecule. Activity is downregulated *in vivo* by autophosphorylation of Tyr⁵²⁷ near the carboxyl terminus of chicken Src (Tyr⁵³⁰

on human c-Src, Tyr⁵³⁴ on mouse c-Src, and Tyr⁵²⁵ on frog c-Src). Phosphorylation at Tyr⁵²⁷ is primarily intramolecular and induces the kinase to form an inactive conformation.^{6,7} Receptor protein tyrosine phosphatase- α (RPTP α) was recently identified as a physiological upstream activator of Src-family kinases.

Autophosphorylation of chicken c-Src also occurs on Tyr⁴¹⁶ (Tyr⁴¹⁴ on frog c-Src, Tyr⁴¹⁸ on human c-Src, and Tyr⁴²³ on mouse c-Src) and is readily demonstrated *in vitro*. Phosphorylation of Tyr⁴¹⁶ 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 Tyr²¹³ (Tyr²¹⁵ on human c-Src, Tyr²¹¹ on frog c-Src, and Tyr²²⁰ 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 downregulation induced by phosphorylation at Tyr⁵²⁷.

Reagent

Anti-phospho-Src (pTyr⁴¹⁸) is supplied as a solution in Dulbecco's phosphate-buffered saline (without Mg²⁺ and Ca²⁺), pH 7.3, with 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 hazardous and safe handling practices.

Storage/Stability

Store at -20 C. For continuous use, store at 2-8 C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not

recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

A recommended working dilution of 1:1000 is determined by immunoblotting using human platelets or NIH-3T3 cells. Data demonstrate that only phosphopeptide corresponding to the region containing Tyr⁴¹⁸ blocks the antibody signal, which confirms the specificity of the Anti-phospho-Src (pTyr⁴¹⁸) for this phosphorylated residue

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

References

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