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ProductInformation

ANTI-phospho-STAT5A/B
(pTyr$^{694/699}$)
Developed in Rabbit
IgG Fraction of Antiserum

Product Number **S 5058**

Product Description

Anti-phospho-STAT5A/B is developed in rabbit using a synthetic peptide LAKAVDG [pY] VKPQ, in which p6 corresponds to phospho-tyrosine 694 of human STAT5 as immunogen. The antibody is rabbit immunoaffinity purified IgG.

Anti-phospho-STAT5A/B reacts tyrosine-694/tyrosine-699 phosphorylated STAT5A/B protein (~95 kDa). It reacts with mouse, rat, human, and bovine phospho-STAT5A/B. An additional unknown protein is detected at approximately 200 kDa. The antibody may be used for the detection of phospho-STATA/B by immuno-blotting and dot blot.

STATs (signal transducers and activators of transcription) are a family of transcription factors that are activated by the JAK family of kinases or by receptor tyrosine kinases. When cells encounter various extracellular ligands, such as interferons and EGF, the STATs promote rapid induction of genes.[1-3]

The STAT proteins are highly conserved at the N-terminal, but have divergent C-terminals, which are thought to be essential for their selective activity. Seven STAT proteins have been described (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6) and range in MW from 84-113 kDa. STATs 1, 3, 4, 5A and 5B have between 750 and 795 amino acid residues, whereas STATs 2 and 6 have approximately 850 amino acid residues.[2,4] Phosphorylation on a single tyrosine located around residue 700 in each protein is required for STAT activation.[1,2] In several forms of human leukemia, STAT5 is constitutively phosphorylated.

Activation of the JAK/STAT pathway begins with ligand (such as Interferon-α) binding to receptor on the plasma membrane and activation of certain members of the JAK tyrosine kinase family. JAKs are associated with the intracellular tail of many cytokine receptors. Receptors to which JAKs are bound are often referred to as cytokine receptors. Their ligands include interferon-α, β, and γ; interleukins (IL) 2-7, 10-13, and 15; and erythropoietin, growth hormone, prolactin, thrombopoietin, and other polypeptides. STAT5 can be activated by a variety of different agents including IL-2, IL-3, IL-7, IL-15, prolactin, growth hormone, erythropoietin and GM-GSF. IL-2 rapidly activates STAT5 in peripheral blood lymphocytes (PBLs). Both STAT3 and STAT5 are activated in phytohemagglutin stimulated PBLs. Ligand-induced dimerization of the receptor results in the reciprocal tyrosine phosphorylation (activation) of the associated JAK. JAK then phosphorylates tyrosine residues on the cytoplasmic tail of the receptor. These phophorylated tyrosines function as docking sites for the SH2 domains of the STAT proteins. Thus, STATs are recruited to the receptor. JAK then catalyzes the tyrosine phosphorylation of the receptor-bound STAT. The phosphorylated STAT molecules then rapidly form homo- or heterodimers. Dimers or heterodimers, but not monomers are competent to bind DNA. The known DNA binding heterodimers are STAT1:2 and STAT1:3.[2] The heterodimer STAT1:2 requires a protein termed p48, a member of the interferon regulatory factor-1 (IRF-1) family of proteins,[6] to become the DNA binding protein ISGF3 (interferon-stimulated growth factor 3). STAT homodimers that bind DNA include STATs 1,3,4, 5 (STAT5A and STAT5B interact in a manner equivalent to a heterodimer), and 6.[2,3,5] STAT2:2 dimers form sparingly in the absence of STAT1 and bind DNA weakly,[7] as do STAT2:3 heterodimers.

Homo- or heterodimers of the STATs translocate to the nucleus, where they either directly interact with promoter elements (gamma-activated sequence or GAS motifs) or combine with a DNA-binding protein (interferon stimulable response element or ISRE motifs). STATs activate distinct target genes despite having similar DNA binding preferences.[2, 8-12]

Selective gene activation by the various STATs may be attributed to differential STAT dimer binding to DNA. Cooperative binding to neighboring sites of two or more STAT dimers enables the STAT proteins to recognize variations of the consensus site. These sites can be specific for the different STAT proteins and may function to direct selective transcriptional activation.

SOCS (suppressor of cytokine signaling) proteins are induced in response to cytokine and suppress signal transduction in two ways. SOCS-1 appears to bind directly to JAKs and inhibit their catalytic activity, and CIS appears to bind to activated receptors and prevent docking of signaling intermediates. SHP-1 suppresses the signal by dephosphorylating either JAKs or the activated receptor subunits, depending on the specific pathway that is activated. PIAS (protein inhibitor of activated STAT) family members inactivate STAT dimers by an unknown mechanism. Activated STAT dimers are probably also downregulated by degradation and dephosphorylation by unknown phosphatases.[13]

Reagent
The antibody is supplied as a IgG fraction solution in 0.1 M Tris-glycine, pH 7.4, containing 0.15 M NaCl, and 0.05% sodium azide before the addition of glycerol to 30%.

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. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile
By immunoblotting, a working antibody dilution of 1:500-1:1,000 is recommended using a PDGF-treated mouse NIH3T3 cell lysate, anti-rabbit IgG conjugated to peroxidase and enhanced chemiluminescence.

By dot blot, a working antibody dilution of 1:500-1:2,000 is recommended to detect the peptide containing hhospho-STAT5A/B (Tyr694/699).

Note: In order to obtain the best results and assay sensitivity in various techniques and preparations, we recommend determining the optimal working dilution by titration.

References

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