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ProductInformation

ANTI-STAT5A
Developed in Rabbit, IgG Fraction of Antiserum

Product Number **S6058**

Product Description

Anti-STAT5A is developed in rabbit using a peptide sequence ([C]RLSPPAGLFTSARGSLS) corresponding to human STAT5A (amino acids 778-794) conjugated to KLH as immunogen. The antibody is purified using protein A chromatography.

Anti-STAT5A reacts specifically with human STAT5A (95 kD). It also reacts with mouse STAT5A.

Anti-STAT5A may be used for the detection of STAT5A by various immunoassays including immunoblotting, and immunoprecipitation. The antibody cannot supershift STAT5A.

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 their 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]

Reagents

The product is supplied as IgG fraction in 0.1 M tris-glycine buffer, pH 7.4, containing 0.15 M sodium chloride, 30% glycerol, and 0.05% sodium azide as a preservative.

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 0 °C to –20 °C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1µg/µl total cell protein in a microcentrifuge tube with PBS (Product No. P3813).
2. Add 4 µg of anti-STAT5A to 500 µg - 1mg cell lysate.
3. Gently rock the reaction mixture at 4 °C overnight.
4. Capture the immunocomplex by adding 100 µl of a washed (in PBS) 1:1 slurry of Protein A-Agarose beads (50 µl packed beads) (Product No. P2545).
5. Gently rock reaction mixture at 4 °C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 50 µl 2X Laemmli sample buffer. The agarose beads can be frozen for later use.
8. Suspend the agarose beads in Laemmli sample buffer and boil for 5 minutes. The beads are pelleted by a microcentrifuge pulse. SDS-PAGE and subsequent immunoblotting analysis may be performed on a sample of the supernatant.

Lysis Buffer:
50 mM Tris-HCl, pH 7.4, containing 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM PMSF, 1 µg/ml each aprotinin, leupeptin, pepstatin, 1 mM Na_3VO_4, and 1 mM NaF.

Product Profile

Working concentration is 1 µg/ml by immunoblotting using human A431, and mouse 3T3 and CTLL cell lysates, anti-rabbit IgG conjugated to peroxidase and enhanced chemiluminescence. For immunoprecipitation, 4 µg will immunoprecipitate STAT5A from 0.5 – 1 mg of a mouse 3T3 cell lysate.

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

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