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Product Information

ANTI-STAT3

Developed in Rabbit
IgG Fraction of Antiserum

Product Number **S 5933**

Product Description

Anti-STAT3 is developed in rabbit using a GST fusion protein corresponding to amino acids 688-722 of human STAT3 (RPESQEHPEADPGSAAPYLKTKFICV-TPTTCSNTI), expressed in bacteria, as immunogen. The sequence is identical in mouse and rat STAT3. The first 28 amino acids are identical to mouse STAT3B. The antibody is purified using protein A chromatography.

Anti-STAT3 reacts specifically with human STAT3 (92 kDa). It also reacts with mouse and rat. Using high concentrations or long exposures, it may detect an additional 66 kDa band. Anti-STAT3 may be used for the detection of STAT3 by various immunochemical techniques including immunoblotting, immunocytochemistry, and immunoprecipitation.

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} STAT 3 is activated by tyrosine phosphorylation at amino acid 704 in cells treated with IL-6 or EGF, but it is not phosphorylated after treatment with interferon-gamma. In addition, stimuli such as elevation of cAMP and intercellular calcium, and

activation of protein kinase C lead to phosphorylation of STAT3 on serine-727. In chronic lymphocyte leukemia, STAT3 is constitutively phosphorylated on serine-727. The phosphorylation of STAT3 on serine-727 in addition to its tyrosine phosphorylation on tyrosine-704 allows the integration of signals generated from diverse signaling pathways.

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 2-7, 10-13, and 15; and erythropoietin, growth hormone, prolactin, thrombopoietin, and other polypeptides. 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 phosphorylated 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 stimutable 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

Anti-STAT3 is supplied as IgG fraction in 0.1 M tris-glycine, pH 7.4, containing 0.15 M NaCl, and 0.05 % sodium azide.

Protein concentration is approximately 1 mg/ml by Bradford.

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

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.

Procedure

Immunoprecipitation

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1 µg/µl total cell protein in a microcentrifuge tube with PBS (Product No. P 3813).
2. Add 4 µg of anti-STAT3 to the cell lysate (500 µg-1mg).
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. P 2545).
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 Na₃VO₄, and 1 mM NaF.

Immunocytochemistry

1. Plate approximately 200 µl of a cell suspension into each well of a slide. Incubate 24 hours in a 37 °C. CO₂ incubator.
2. Wash the cells 3 X for 5 min. with PBS. Do not shake cells.
3. Add fixative (95% ethanol, 5% acetic acid) for 10 min. at room temperature.
4. Wash the cells with PBS, 2 X for 15 min. Do not shake cells.
5. Add 400 µl PBS containing 8% BSA and incubate 30 min. at room temperature.
6. Wash cells with PBS for 15 min.
7. Incubate the cells with 10 µg/ml of anti-STAT3 in PBS containing 1% BSA and incubate 1 hr. at room temperature.
8. Wash the cells 2 X with PBS for 5 min.
9. Incubate the cells with a 1:100 dilution of anti-rabbit IgG conjugated with FITC (Sigma Product No. F9887) in PBS containing 1% BSA for 1 hr. at room temperature.

10. Wash the cells 3 X with PBS for 30 min.
11. Examine the cells under a fluorescent microscope.

Product Profile

By immunoblotting, the recommended working antibody concentration is 0.5 - 2 µg/ml using RIPA lysates from EGF stimulated human A431, mouse WEHI, and rat L6 cells. Detection was done using anti-rabbit IgG conjugated to peroxidase and enhanced chemiluminescence.

By immunocytochemistry, the recommended working antibody concentration is at least 10 µg/ml using A431 cells fixed with 95% ethanol and 5% acetic acid.

By immunoprecipitation, 4 µg will immunoprecipitate STAT3 from 0.5-1 mg of an EGF stimulated human A431 cell lysate.

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

References

1. Darnell Jr., J.E., et al., Science, **264**, 1415 (1994).
2. Schindler, C., and Darnell, J.E., Ann. Rev. Biochem., **64**, 621 (1995).
3. Leaman, D.W., et al., FASEB J., **10**, 1578 (1996).
4. Hou, J., Science, **265**, 1701 (1994).
5. Ihle, J.N., et al., Ann. Review Immunol., **13**, 369 (1995).
6. Kanno, Y., et al., Mol. Cell. Biol., **13**, 3951 (1993).
7. Bluysen, et al., Proc. Natl. Acad. Sci., USA, **92**, 5645 (1995).
8. Wakao, H., et al., EMBO J., **13**, 2182 (1994).
9. Horvath, C.M., et al., Genes Dev., **9**, 984 (1995).
10. Xu, X., et al., Science, **273**, 794 (1996).
11. Mikita, T., et al., Mol. Cell. Biol., **16**, 5811 (1996).
12. Seidel, H.M., et al., Proc. Natl. Acad. Sci., USA, **92**, 3041 (1995).
13. Starr, R., and Hilton, D.J., Bioessays, **21**, 47 (1999).

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