# ProductInformation

**MONOCLONAL ANTI-PHOSPHO-STAT3 (Phosphotyrosine 705), CLONE 9E12 Purified Mouse Immunoglobulin**

Product Number **S 4183**

## Product Description

Monoclonal Anti-Phospho-STAT3 (mouse IgG1 isotype) is derived from the 9E12 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from mice immunized with a synthetic peptide containing the AP[pY]LK motif of human STAT3 conjugated to KLH. The antibody is purified from ascites fluid using thiophilic adsorption and subsequent size exclusion chromatography.

Monoclonal Anti-Phospho-STAT3 reacts specifically with human tyrosine-705 phosphorylated STAT3 protein (92 kDa). It also reacts with mouse phospho-STAT3.

Monoclonal Anti-Phospho-STAT3 may be used for detection of phospho-STAT3 using various immunoassays including immunoblotting 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-705, 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 λ; interleukins (IL) 2-7, 10-13, and 15; and erythropoietin, growth hormone, prolactin, thromopoietin, 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 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 purified immunoglobulin in phosphate buffered saline, pH 7.4, containing 30% glycerol, and 0.035% sodium azide (see MSDS)* as a preservative.

Protein concentration is approximately 0.7 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**
Store at 0 °C to −20 °C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

**Product Profile**
Working concentration is at least 0.1 µg/ml by immunoblotting using human SK-N-MC cells stimulated with CNTF, anti-mouse IgG conjugated to peroxidase and enhanced chemiluminescence. For immunoprecipitation, 1-10 µg/ml will immunoprecipitate phosphoSTAT3 from a lysate of 1 x $10^6$ vanadate-treated human A431 cells.

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

**References**

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