



MONOCLONAL ANTI-JAK3, CLONE B32-32
Purified Mouse Immunoglobulin

Product Number **J3252**

Product Description

Monoclonal Anti-JAK3 (mouse IgG2b isotype) is derived from the B32-32 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from mice immunized with a synthetic peptide (KAVDG [pY] VKPQIK), corresponding to amino acids 1076-1100 of mouse JAK3. The antibody is purified from ascites fluid using protein A chromatography.

Monoclonal Anti-JAK3 reacts specifically with mouse JAK3 (125 kD). It also reacts with human JAK3.

Anti-JAK3 may be used for the detection of JAK3 by immunoprecipitation.

The Janus Kinase (JAK) family is a protein tyrosine kinase (PTK) family involved in cytokine signaling, activated by type I and type II cytokine receptors. It plays a pivotal role in the signal transduction process mediated by cytokines. These kinases appear to transduce signals via their substrates, which modulate programs of gene expression specific to the respective signals. The activation of JAKs is associated with rapid tyrosine phosphorylation of the Signal Transducers and Activators of Transcription (STAT) proteins. At present, the JAK family includes JAK1, JAK2, JAK3 and Tyk2.

The JAKs are 130-kDa proteins that lack SH2/SH3 domains and contain two kinase domains, an active domain and a second kinase-like domain. JAK1, JAK2 and TYK2 are ubiquitous, whereas JAK3 is predominantly expressed in T lymphocytes.

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. Receptors to which JAKs are bound are often referred to as cytokine receptors. JAKs are associated with the intracellular tail of many cytokine receptors. Their ligands include interferon- α , β , and λ ; interleukins (IL) 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

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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.^{1,2}

SOCS (suppressor of cytokine signaling) proteins are induced in response to cytokine^{3,4} and suppress signal transduction in two ways. SOCS-1 appears to bind directly to JAKs and inhibit their catalytic activity,^{3,5,6} and CIS, a member of the SOCS family (cytokine-inducible SH2), appears to bind directly to activated receptors and prevent docking of signaling intermediates.^{7,8} The phosphatase SHP-1 can also suppresses the signal by dephosphorylating either JAKs or the activated receptor subunits, depending on the specific pathway that is activated.

Besides activating STATs, activated JAKs can bind Shc proteins that recruit Grb-2-SOS complexes, thereby initiating the Ras-MAP kinase pathway. Activated JAKs can also bind insulin receptor substrate (IRS) proteins that are thought to regulate metabolic events in the cell.⁹

Reagents

The product is supplied as purified mouse immunoglobulin in 0.07 M tris-glycine buffer, pH 7.4, containing 0.105 M NaCl, 30% glycerol and 0.035% sodium azide.

Protein concentration is approximately 0.4 mg/ml by Bradford.

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 2 μ g/ μ l total cell

protein in a microcentrifuge tube with PBS (Product No. P3813).

2. Add 5 µg of Monoclonal Anti-JAK3 to 1 – 2 mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 30 µl of a washed (in PBS) 1:1 slurry of Protein G-Agarose beads (15 µl packed beads) (Product No. P2294).
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. Pellet the beads 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.

Product Profile

Working concentration for immunoprecipitation is 5 µg to immunoprecipitate JAK3 from 1 – 2 mg of a human TF1 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.

References

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