ProductInformation

ANTI-JAK1 (797-817)
Developed in Rabbit, Whole Antiserum

Product Number **J3377**

Product Description

Anti-JAK1 is developed in rabbit using a peptide sequence (KTLIEKERFYESRCRPVTPSC) corresponding to amino acids 797-817 of human JAK1 as immunogen. This sequence shares 100% homology with mouse JAK1. The antibody is supplied as whole antiserum.

Anti-JAK1 reacts specifically with human JAK1 (approximately 130 kD) and a degradation product of JAK1 (approximately 110 kD) may also be detected. The antibody also reacts with mouse, rat, rabbit and Chinese hamster. Other species reactivity is unknown.

Anti-JAK1 may be used for the detection of JAK1 by immunoblotting and 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, 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.[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 whole antiserum.

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. Clear the lysate of proteins that non-specifically bind to Protein A and rabbit serum.
   a) Add 5 µl normal rabbit serum (Product No. R9133) to 1-2 mg of lysate. Incubate 1hr to overnight at 4°C.
   b) Add 100 µl of a washed (in PBS) 1:1 slurry of Protein A-Agarose beads (50 µl packed beads) (Product No. P2545). Incubate agarose/serum/lysate for 30 min. at 4°C.
   c) Centrifuge lysate mixture in a microfuge at 900 x g for one min.
   d) Transfer the supernatant fraction to another microcentrifuge tube.
   e) Repeat steps b-f to remove any residual rabbit proteins. The final supernatant fraction is the cleared lysate.
3. Add 5 µl of anti-JAK1 to the cleared lysate.
4. Gently rock the reaction mixture at 4°C overnight.
5. Capture the immunocomplex by adding 100 µl of a washed (in PBS) 1:1 slurry of Protein A-Agarose beads (50 µl packed beads) (Sigma Product No. P2545).
6. Gently rock reaction mixture at 4°C for 1-2 hours.
7. 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.
8. Resuspend the agarose beads in 50 µl 2X Laemmli sample buffer. The agarose beads can be frozen for later use.
9. 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 Na_3VO_4, and 1 mM NaF.

Product Profile

Working dilution is 1:500 by immunoblotting using immunoprecipitated JAK1 from mouse CTLL lymphocytes, anti-rabbit IgG conjugated to peroxidase and enhanced chemiluminescence. The amount of JAK1 in cell lines tends to extremely low. It is recommended to first concentrate the JAK1 by immunoprecipitation prior to immunoblotting.
For immunoprecipitation, 5 µl will immunoprecipitate JAK1 from 0.5 – 1 mg of a mouse CTLL 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

1. Darnell Jr., J.E., Science, **277**, 1630 (1997)
2. Pellegrini, S., and Dusanter-Fourt, I., Eur. J. Biochem., **248**, 615 (1997).
3. 20. Star, et al., Nature, **387**, 917 (1997).
4. Adams, T.E., et al., J. Biol. Chem., **273**, 1285 (1998).
5. Endo, T.A., et al., Nature, **387**, 921 (1997).
6. Naka, T., et al., Nature, **387**, 924 (1997).
7. 26. Yoshimura, A., et al., EMBO J., **14**, 2816 (1995).
8. Matsumoto, A., et al., Blood, **89**, 3148 (1997).
9. Giorgetti-Peraldi, S., et al., Eur J Biochem, **1;234(2)**, 656 (1995).

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