BP7223

OriGene Technologies Inc.

9620 Medical Center Drive, Ste 200
Rockville, MD 20850
UNITED STATES
Phone: +1-858-888-7900
Fax: +1-858-888-7904
US-info@acris-antibodies.com

OriGene EU

Acris Antibodies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info@acris-antibodies.com

Polyclonal Antibody to STAT1 alpha - Aff - Purified

Alternate names: DKFZP686B04100, ISGF-3, P84, P91, STAT1-alpha, STAT1a, STAT91, Signal Transducer And Activator Of Transcription 1 91kDa, Transcription Factor ISGF-3 Components P91/P84

Catalog No.: BP7223

Quantity: 0.1 mg

Background: The signal transducer and activator of transcription (STAT) family of transcription factors is composed of seven family members (STATs 1, 2, 3, 4, 5A, 5B and 6) that are involved in many cell processes (apoptosis, anti-apoptosis, cell differentiation and proliferation), depending upon the cell type. STATs are activated by ligand binding to cytokine receptors and other receptors associating with Janus kinase (JAK) family members that phosphorylate STAT proteins, causing them to assemble and translocate to the nucleus. In the nucleus, phosphorylated STAT protein complexes bind to DNA and regulate the transcription of particular genes. STAT1α (91 kDa) participates in a signaling pathway which is initiated by IFN-γ. The STAT1α antibody does not recognize STAT1β, which is missing the C-terminal 38 amino acids present in STAT1α. STAT1α is useful as a positive control for measuring total STAT1α protein levels.

Host: Rabbit

Immunogen: Chemically synthesized peptide derived from the C-terminal region of mouse STAT1α

Format:
State: Liquid Ig fraction
Purification: Epitope-specific affinity chromatography
Buffer System: Dulbecco's phosphate buffered saline (without Mg2+ and Ca2+), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier, containing 0.05 % sodium azide

Applications: Western blot (0.1 - 1.0 µg/ml).
Positive Control Used: NIH3T3 cells; 3T3-L1 adipocytes.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody detects STATalpha.
Species: Human, Mouse.
Other species not tested.

Storage: Store at 2 - 8 °C up to one week or (in aliquots) at -20 °C for longer. Avoid repeated freezing and thawing.
Centrifuge vial before opening.
Shelf life: one year from despatch.

General Readings: Choudhury, G.C. (2004) A linear signal transduction pathway involving phosphatidylinositol 3-kinase, protein kinase Cepsilon, and MAPK in mesangeal cells regulates interferon-gamma-induced STAT1alpha transcriptional activation. J. Biol. Chem. 279(26):27399-27409.

Burysek, L., et al. (2002) The serine protease plasmin triggers expression of MCP-1 and CD40 in human primary monocytes via activation of p38 MAPK and Janus kinase (JAK)/STAT signaling pathways. J. Biol. Chem. 277(36):33509-33517.
Chen, G., et al. (2001) Expression of the transcription factor STAT-1α in insulinoma cells protects against cytotoxic effects of multiple cytokines. J. Biol. Chem. 276(1):766-772.
Subramaniam, P.S., et al. (2000) The COOH-terminal nuclear localization sequence of interferon γ regulates STAT1α nuclear translocation at an intracellular site. J. Cell Sci. 113:2771-2781.
Singh, K., et al. (1996) Regulation of cytokine-inducible nitric oxide synthase in cardiac myocytes and microvascular endothelial cells. Role of extracellular signal-regulated kinases 1 and 2 (ERK1/ERK2) and STAT1α. J. Biol. Chem. 271(2):1111-1117.
Hague, S.J., et al. (1995) Roles of protein-tyrosine phosphatases in STAT1α-mediated cell signaling. J. Biol. Chem. 270(43):25709-25714.

Protocols:

Western Blotting Procedure

1. Lyse approximately 10e7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifugedat 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH2O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) for one hour at room temperature or overnight at 4°C.
12. Incubate the blocked blot with primary antibody at 0.1-1.0 µg/mL in Tris buffered saline supplemented with 3% non-fat dried milk and 0.1% Tween 20 overnight at 4°C or for two hours at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat F(ab)2 anti-rabbit IgG alkaline phosphatase conjugate or goat F(ab)2 anti-rabbit IgG horseradish peroxidase conjugate in conjunction with your chemiluminescence reagents and instrumentation.

Cell Lysis Buffer Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM Na4P2O7
2 mM Na3VO4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO)
or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma Cat. # P2714 may be used)

Transfer Buffer Formulation:
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add 1 mL 10% SDS.
Cool to 4°C prior to use.

Tris Buffered Saline Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer Formulation:
100 mL Tris buffered saline
5 gm BSA
0.1 mL Tween 20

Pictures:

Western Blotting Lysates of NIH3T3 cells stimulated with 50 ng/mL PDGF for 5 minutes were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. The membrane was blocked with a 5% BSA-TBST buffer for one hour at room temperature, and then incubated with 0.50 µg/mL STAT1α antibody for two hours at room temperature in a 3% milk-TBST buffer. After washing, the membrane was incubated with goat F(ab')2 anti-rabbit IgG HRP-conjugate and signals were detected using the Pierce SuperSignalTM method. The data show total level of STAT1α present in these cells stimulated under this condition.