Rabbit (polyclonal) Anti-STAT 2, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number:	44-362G (10 mini-blot size)
Lot Number:	See product label
Volume:	100 µL
Form of Antibody:	Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier.
Preservation:	0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)
Purification:	Purified from rabbit serum by epitope-specific affinity chromatography.
Immunogen:	The antiserum was produced against a chemically synthesized peptide derived from the C-terminal region of human STAT2.
Target Summary:	STATs (signal transducers and activators of transcription) were originally discovered as two proteins (STAT 1 and 2) involved in IFN-α and IFN-γ signal transduction. Since their initial identification, 5 more STAT proteins have been discovered (STAT 3, 4, 5a, 5b and 6). STATs undergo tyrosine phosphorylations in response to growth factor or cytokine signaling (in some cases mediated by JAK kinases [Janus Kinases 1, 2 and 3]), resulting in dimerization and translocation of STAT proteins to the nucleus. Phosphorylation at serine residues on certain STATs (STAT 1α, 3, 4 and 5) has also been reported, and appears to be required for the maximal activation of these proteins. STAT 2 (113 kDa) is involved in interferon alpha transcription and STAT1 phosphorylation.
Reactivity:	Human STAT2. Other species have not been tested.
Applications:	This antibody has been used in Western blotting. Other applications have not been tested.
Suggested Working Dilutions:	For Western blotting applications, we recommend using the antibody at a 1:1,000 starting dilution. The optimal antibody concentration should be determined empirically for each specific application.
Storage:	Store at –20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at –20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.
Expiration Date:	Expires one year from date of receipt when stored as instructed.
Positive Control Used:	HeLa cells

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Related Products:

Antibodies:

STAT1α, Cat. # 44-360G
STAT1 [pY^{701}] mo, Cat. # 44-376G
STAT1 [pS^{727}], Cat. # 44-382G
STAT3, Cat. # 44-364G
STAT3 [pY^{705}], Cat. # 44-380G
STAT3 [pS^{727}], Cat. # 44-384G
STAT4, Cat. # 44-366G
STAT5, Cat. # 44-368G
STAT5α [pY^{694}], Cat. # 44-390G
STAT6, Cat. # 44-372G
JAK/STAT Sampler Pack, Cat. # 44-429G

ELISAs:

STAT1 [pY^{701}], Cat. # KHO0271
STAT3 [pY^{705}], Cat. # KHO0481

References:

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Frahm, T., H. Hauser and M. Koster (2006) IFN-type-I-mediated signaling is regulated by modulation of STAT2 nuclear export. J. Cell Sci. 119(Pt. 6):1092-1104.

Brierley, M.M. and E.N. Fish (2005) Functional relevance of the conserved DNA-binding domain of STAT2. J. Biol. Chem. 280(13):13029-13036.

Banninger, G. and N.C. Reich (2004) STAT2 nuclear trafficking. J. Biol. Chem. 279(38):39199-39206.

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Stewart, M.D. (2002) Roles of STAT1, STAT2, and interferon regulatory factor-9 (IRF-9) in interferon Tau regulation of IRF-1. Biol. Reprod. 66(2):393-400.

Zhou, Y., et al. (2001) Interferon alpha induction of STAT1 and STAT2 and their prognostic significance in carcinoid tumors. Oncology. 60(4):330-338.

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Johnson, L.R., et al. (1999) EGF induces nuclear translocation of STAT2 without tyrosine phosphorylation in intestinal epithelial cells. Am. J. Physiol. 276(2 Pt. 1):C419-C425.

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Bluyssen, H.A. and D.E. Levy (1997) STAT2 is a transcriptional activator that requires sequence-specific contacts provided by STAT1 and p48 for stable interaction with DNA. J. Biol. Chem. 272(7):4600-4605.

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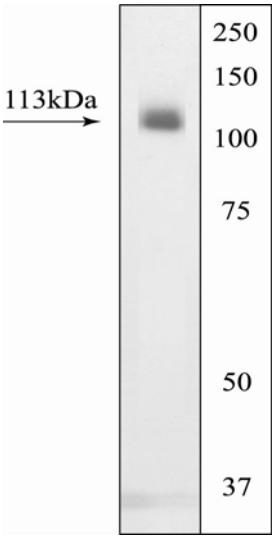

Western Blot

Extracts of HeLa cells were resolved by SDS-PAGE on a 4-20% Tris-glycine gel and transferred to PVDF. The membrane was blocked with a 5% Milk-BSA-TBST buffer for one hour at room temperature, then incubated with the STAT2 antibody for two hours at room temperature in a 3% BSA-TBST buffer. After washing, the membrane was incubated with goat $F(ab')_2$ anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and signals were detected using the Pierce SuperSignal™ method.

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Western Blotting Procedure

1. Lyse approximately 10^7 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. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Invitrogen catalog number FNN0011). 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 ultracentrifuged at 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 ddH_2O for 5 minutes.
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.
1. Block the membrane with blocking buffer (formulation provided below) overnight at 4°C or for one hour at room temperature.
11. Incubate the blocked blot with primary antibody at a 1:1,000 starting dilution in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween-20 overnight at 4°C or for two hours at room temperature.
12. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
13. Detect the antibody band using an appropriate secondary antibody, such as goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase conjugate (catalog number ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (catalog number ALI4404) 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 $Na_4P_2O_7$
2 mM Na_3VO_4
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)
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma catalog number 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 non-fat dried milk
0.1 mL Tween 20

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