

Datasheet for 600-401-A93S**STAT2 phospho Y690 Antibody****Overview**

Description:	Anti-STAT2 pY690 (RABBIT) Antibody - 600-401-A93S
Item No.:	600-401-A93S
Size:	25 µL
Applications:	ELISA, IHC, WB
Reactivity:	Human, Mouse
Host Species:	Rabbit

Product Details

Background:	This antibody is designed, produced, and validated as part of a collaboration between Rockland and the National Cancer Institute (NCI) and is suitable for Cancer, Immunology and Nuclear Signaling research. STAT2 is a member of the STAT family of transcription factors. Unlike other STATs, STAT2 is unique as it can only be activated by interferons (IFNs). STAT2 is a critical component in mediating many IFN-stimulated biological activities including antiproliferation and antiviral responses. Upon IFN treatment, STAT1 and STAT2 become tyrosine phosphorylated, assemble as heterodimers that bind IRF9 to form the ISGF3 complex. This complex translocates to the nucleus, binds to promoters of IFN-stimulated genes and mediates gene transcription. Consequently, mutations in STAT2 or loss of STAT2 expression leads to impairment in IFN signal transduction and gene activation. IFN-alpha is an approved drug for the treatment of several forms of cancer. Yet only a subset of patients who receive IFN-alpha therapy benefit from the treatment. Given that STAT2 is activated by IFNs, it is important to define if the reduced or lack of antitumor effects seen in cancer patients on IFN therapy is due to defects in STAT2 function. Our goal is to identify regions/motifs within the structural domains of STAT2 that not only are essential for the tyrosine phosphorylation of STAT2, but also regulate the antitumor effects of IFN-alpha. Collectively, the results of our studies will emphasize the physiological role of STAT2 in cancer. From a clinical viewpoint, cancer patients who may benefit the most from receiving IFN-alpha therapy can be selected based on their STAT2 function.
Synonyms:	rabbit anti-STAT2 pY690 antibody, phosphorylated STAT2 antibody, STAT-2, STAT 2, Signal transducer and activator of transcription 2, p113
Host Species:	Rabbit
Clonality:	Polyclonal

Format: IgG

Target Details

Gene Name:	STAT2
Reactivity:	Human, Mouse
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to a C-terminus region near phospho Tyrosine 690 of human STAT2 protein.
Purity/Specificity:	This affinity purified antibody is directed against human STAT2 pY690 protein. The product was affinity purified from monospecific antiserum by immunoaffinity chromatography. A BLAST analysis was used to suggest cross-reactivity with STAT2 pY690 protein from human, rat (73%) and mouse (76%), sources based on homology with the immunizing sequence. Reactivity against homologues from other sources is not known.
Relevant Links:	• UniProtKB - P52630 • NCBI - NP_005410.1 • GeneID - 6773

Application Details

Tested Applications:	ELISA, IHC, WB
Application Note:	This affinity purified antibody has been tested for use in ELISA, western blotting, and IHC. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 113 kDa in size corresponding to STAT2 pY690 protein by western blotting in the appropriate cell lysate or extract.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:85,000
IHC:	1:100
WB:	1:500 – 1:1000

Formulation

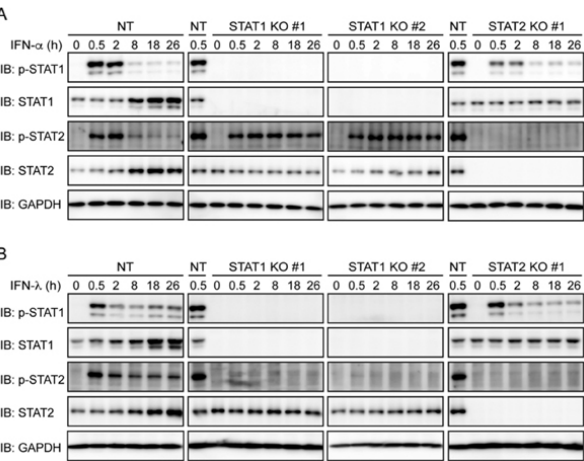
Physical State: Liquid (sterile filtered)

Concentration:	1.15 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

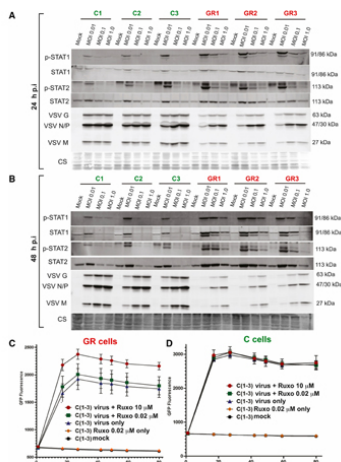
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Expiration:	Expiration date is three (3) months from date of receipt.

Images

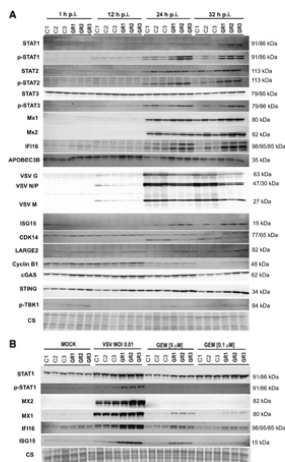


Western Blot
(A,B) NT sgRNA-expressing Huh-7.5 cells, STAT1 knockout (KO) cells (clones #1 and #2), and STAT2 KO cells (clone #1) were treated with IFN-α (1,000 U/ml) (A) or IFN-λ (1,000 U/ml) (B) for the indicated times. Phosphorylation of STAT1 and STAT2 was evaluated by immunoblotting (IB). Fig 7. PMID: 27929099



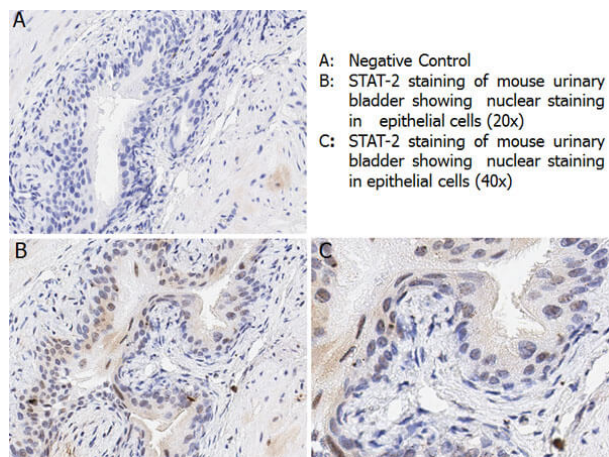
Western Blot

VSV and antiviral protein expression
(A and B) C and GR cells were either mock treated or infected with VSV-ΔM51 at MOIs of 1.0, 0.1, and 0.01. Protein sample were analyzed at 24 h p.i. (A) and 48 h p.i. (B) by western blotting for expression of phospho-STAT1 (p-STAT1), phospho-STAT2 (p-STAT2), STAT1, STAT2, and VSV proteins (G, N/P, and M). Cell line and treatment conditions are indicated above blots. Equal protein loading is indicated by Coomassie blue. (C and D) The effect of ruxolitinib on VSV-ΔM51 replication kinetics based on GFP fluorescence. C and GR cells were either mock treated, treated only with ruxolitinib, treated only with VSV-ΔM51, or treated with both VSV-ΔM51 and ruxolitinib. Cells were infected at MOI of 0.01. Ruxolitinib was added to cells after 1 h VSV incubation period. GFP fluorescence was measured from 1 to 80 h p.i. The data points and error bars shown represent the means and SEM of the means, respectively. Control and GR cell lines 1–3 are combined for each treatment. Fig 5. PMID: 34977342



Western Blot

Time course expression of antiviral proteins in C and GR cells after VSV infection
(A) C and GR cells were infected with VSV-ΔM51 at MOI 0.01. Protein samples were collected at 1, 12, 24, and 32 h p.i. and analyzed by western blotting. Cell lines and time points are indicted above the blots. Equal protein loading is indicated by Coomassie blue. (B) C and GR cells were either mock treated, infected with VSV-ΔM51, or treated with 5 μM gemcitabine or 0.1 μM gemcitabine. Protein samples were collected after 32 h and analyzed by western blotting for expression of ISGs. Cell lines and time points are indicted above the blots. Equal protein loading is indicated by Coomassie blue. Fig 7. PMID: 34977342



Immunohistochemistry

Immunohistochemistry with anti-STAT2 pY690 antibody showing nuclear positivity in epithelial cells of mouse urinary bladder tissue at 20x and 40x (B & C). Staining was performed on Leica Bond system using the standard protocol. Formalin fixed/paraffin embedded tissue sections were subjected to antigen retrieval and then incubated with rabbit anti-STAT2 pY690 antibody 600-401-A93 at 1:100 dilution for 60 minutes. Biotinylated Anti-rabbit secondary antibody was used at 1:200 dilution to detect primary antibody. The reaction was developed using streptavidin-HRP conjugated compact polymer system and visualized with chromogen substrate, 3'3-diamino-benzidine substrate (DAB). The sections were then counterstained with hematoxylin to detect cell nuclei.

Western Blot

Western blot using Rockland Immunochemicals affinity purified anti-Stat2pY690 antibody shows detection of Stat2pY690 protein (arrowhead). Loaded Jurkat cells treated without (left lane) or with (right lane) 1000U/mL of IFN- α for 15 min at 37°C. Primary antibody was used at 1:1,000. Personal Communication, A.Gamero, NCI, Bethesda, MD.



References

- Goad, DW et al. Acquired chemoresistance can lead to increased resistance of pancreatic cancer cells to oncolytic vesicular stomatitis virus. *Molecular Therapy Oncolytics* (2022)
- Yamauchi et al. STAT1 is essential for the inhibition of hepatitis C virus replication by interferon- λ but not by interferon- α . *Scientific Reports* (2016)

Disclaimer

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