# Anti-STAT1 alpha Antibody [SJ01-89]

## ET1606-39

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| **Product Type:** | Recombinant Rabbit monoclonal IgG, primary antibodies |
| **Species reactivity:** | Human, Mouse |
| **Applications:** | WB, IP, FC, IHC-P, IF-Cell, IF-Tissue |
| **Molecular Wt:** | Predicted band size: 87 kDa |
| **Clone number:** | SJ01-89 |

**Description:** Membrane receptor signaling by various ligands, including interferons and growth hormones such as EGF, induces activation of JAK kinases which then leads to tyrosine phosphorylation of the various Stat transcription factors. Stat1 and Stat2 are induced by IFN-α and form a heterodimer which is part of the ISGF3 transcription factor complex. Although early reports indicate Stat3 activation by EGF and IL-6, it has been shown that Stat3β appears to be activated by both while Stat3α is activated by EGF, but not by IL-6. Highest expression of Stat4 is seen in testis and myeloid cells. IL-12 has been identified as an activator of Stat4. Stat5 has been shown to be activated by Prolactin and by IL-3. Stat6 is involved in IL-4 activated signaling pathways. Mutations in the STAT1 molecule can be gain of function (GOF) or loss of function (LOF). Both of them can cause different phenotypes and symptoms. Recurring common infections are frequent in both GOF and LOF mutations. In humans STAT1 has been particularly under strong purifying selection when populations shifted from hunting and gathering to farming, because this went along with a change in the pathogen spectrum. STAT1 loss of function, therefore STAT1 deficiency can have many variants. There are two main genetic impairments that can cause response to interferons type I and III. First there can be autosomal recessive partial or even complete deficiency of STAT1. That causes intracellular bacterial diseases or viral infections and impaired IFN a, b, g and IL27 responses are diagnosed. In partial form there can also be found high levels of IFNg in blood serum. When tested from whole blood, monocytes do not respond to BCG and IFNg doses with IL-12 production. In complete recessive form there is a very low response to anti-viral and antimycotical medication. Second, partial STAT1 deficiency can also be an autosomal dominant mutation; phenotypically causing impaired IFNg responses and causing patients to suffer with selective intracellular bacterial diseases (MSMD).

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| **Immunogen:** | Synthetic peptide within human STAT1 aa 710-750. |
| **Positive control:** | Jurkat cell lysate, A431 cell lysate, HeLa cell lysate, A549 cell lysate, SK-Br-3 cell lysate, SK-MEL-28 cell lysate, HT-29 cell lysate, RAW264.7 cell lysate, C2C12 cell lysate, human colon tissue, human ovary cancer tissue, human spleen tissue, mouse colon tissue, MCF-7. |
| **Subcellular location:** | Cytoplasm, Nucleus. |
| **Database links:** | SwissProt: P42224 Human \| P42225 Mouse |
| **Recommended Dilutions:** | |
| **WB** | 1:1,000-1:5,000 |
| **FC** | 1:50-1:100 |
| **IP** | Use at an assay dependent concentration. |
| **IHC-P** | 1:500-1:2,000 |
| **IF-Cell** | 1:100 |
| **IF-Tissue** | 1:50-1:400 |
| **Storage Buffer:** | 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide. |
| **Storage Instruction:** | Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles. |
| **Purity:** | Protein A affinity purified. |

## Images

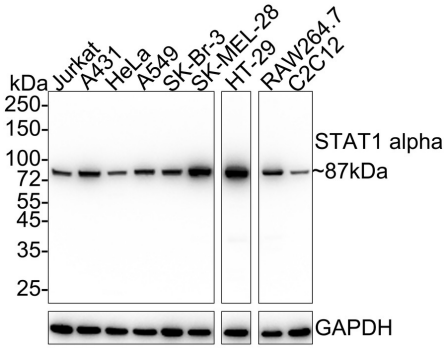


**Fig1:** Western blot analysis of STAT1 alpha on different lysates with Rabbit anti-STAT1 alpha antibody (ET1606-39) at 1/1,000 dilution.

Lane 1: Jurkat cell lysate
Lane 2: A431 cell lysate
Lane 3: HeLa cell lysate
Lane 4: A549 cell lysate
Lane 5: SK-Br-3 cell lysate
Lane 6: SK-MEL-28 cell lysate
Lane 7: HT-29 cell lysate
Lane 8: RAW264.7 cell lysate
Lane 9: C2C12 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 87 kDa
Observed band size: 87 kDa

Exposure time: 14 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1606-39) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

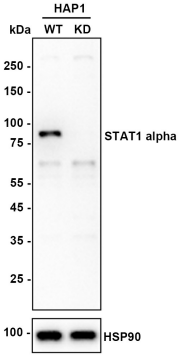


**Fig2:** Western blot analysis of STAT1 alpha on different lysates with Rabbit anti-STAT1 alpha antibody (ET1606-39) at 1/5,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-STAT1 alpha KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 87 kDa
Observed band size: 87 kDa

Exposure time: 60 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1606-39) at 1/5,000 dilution was used in K1803 at

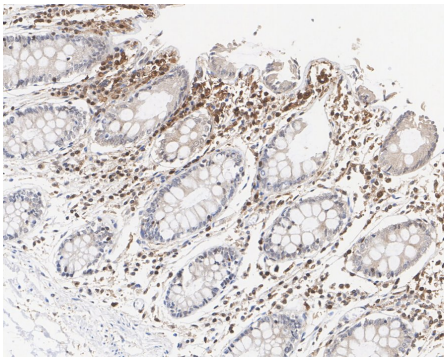

**Fig3:** Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-STAT1 alpha antibody (ET1606-39) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with $ddH_2O$ and PBS, and then probed with the primary antibody (ET1606-39) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

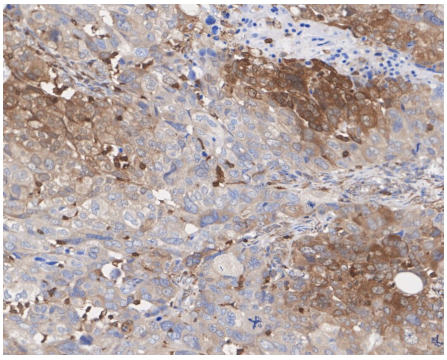

**Fig4:** Immunohistochemical analysis of paraffin-embedded human ovary cancer tissue with Rabbit anti-STAT1 alpha antibody (ET1606-39) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with $ddH_2O$ and PBS, and then probed with the primary antibody (ET1606-39) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

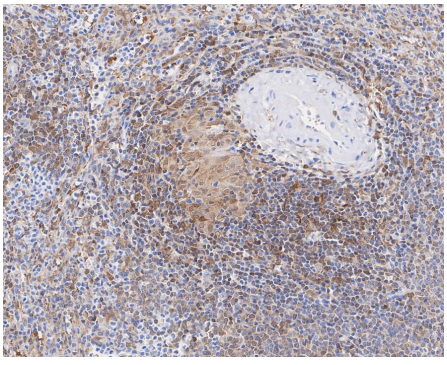

**Fig5:** Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-STAT1 alpha antibody (ET1606-39) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with $ddH_2O$ and PBS, and then probed with the primary antibody (ET1606-39) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

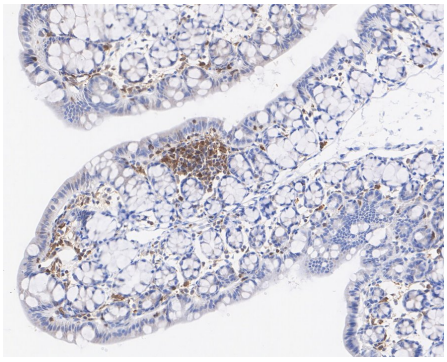

**Fig6:** Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-STAT1 alpha antibody (ET1606-39) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with $ddH_2O$ and PBS, and then probed with the primary antibody (ET1606-39) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

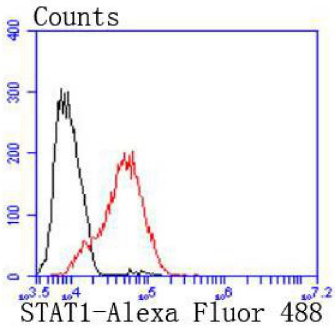


**Fig7:** Flow cytometric analysis of STAT1 alpha was done on MCF-7 cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1606-39, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1,000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

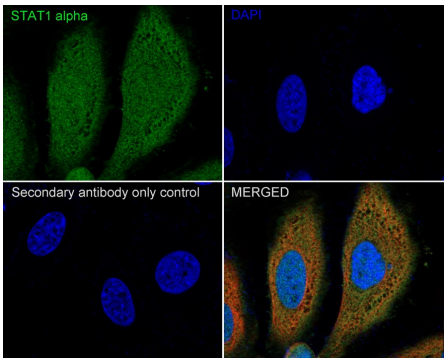


**Fig8:** Immunocytochemistry analysis of HeLa cells labeling STAT1 alpha with Rabbit anti-STAT1 alpha antibody (ET1606-39) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-STAT1 alpha antibody (ET1606-39) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

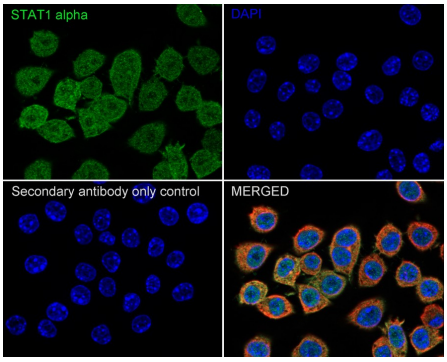


**Fig9:** Immunocytochemistry analysis of RAW264.7 cells labeling STAT1 alpha with Rabbit anti-STAT1 alpha antibody (ET1606-39) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-STAT1 alpha antibody (ET1606-39) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.



## Background References

1. Shakya R et al. Hypomethylating therapy in an aggressive stroma-rich model of pancreatic carcinoma. Cancer Res 73:885-96 (2013).
2. Syu LJ et al. Transgenic expression of interferon- in mouse stomach leads to inflammation, metaplasia, and dysplasia. Am J Pathol 181:2114-25 (2012).