Anti-STAT1 Antibody [SD20-75]

ET1612-22

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 87/83 kDa
Clone number:	SD20-75

Description: STAT1 is involved in upregulating genes due to a signal by either type I, type II, or type III interferons. In response to IFN-γ stimulation, STAT1 forms homodimers or heterodimers with STAT3 that bind to the GAS (Interferon-Gamma-Activated Sequence) promoter element; in response to either IFN-α or IFN-β stimulation, STAT1 forms a heterodimer with STAT2 that can bind the ISRE (Interferon-Stimulated Response Element) promoter element.[12] In either case, binding of the promoter element leads to an increased expression of ISG (Interferon-Stimulated Genes).

Immunogen: Synthetic peptide within Human STAT1 alpha aa 1-50 / 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, MCF7 cell lysate, HT-29 cell lysate, NIH/3T3 cell lysate, RAW264.7 cell lysate, C2C12 cell lysate, L6 cell lysate, Hela, MCF-7, SKOV-3, human colon tissue, mouse colon tissue, mouse spleen tissue, rat colon tissue, NIH/3T3, PC-12.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P42224 Human | P42225 Mouse
Entrez Gene: 25124 Rat

Recommended Dilutions:

WB	1:1,000-1:5,000
IF-Cell	1:50-1:200
IF-Tissue	1:200
IHC-P	1:500-1:2,000
FC	1:1,000
IP	1-2μg/sample

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

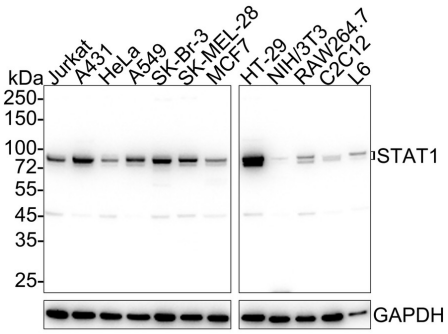

Fig1: Western blot analysis of STAT1 on different lysates with Rabbit anti-STAT1 antibody (ET1612-22) at 1/1,000 dilution.

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: MCF7 cell lysate
Lane 8: HT-29 cell lysate
Lane 9: NIH/3T3 cell lysate
Lane 10: RAW264.7 cell lysate
Lane 11: C2C12 cell lysate
Lane 12: L6 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 87/83 kDa
Observed band size: 87/83 kDa

Exposure time: 10 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 (ET1612-22) 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.

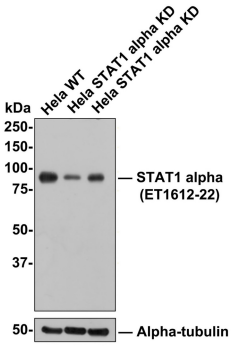

Fig2: All lanes: Western blot analysis of STAT1 alpha with anti-STAT1 alpha antibody (ET1612-22) at 1:500 dilution.
Lane 1: Wild-type Hela whole cell lysate (10 µg).
Lane 2/3: STAT1 alpha knockdown Hela whole cell lysate (10 µg).

ET1612-22 was shown to specifically react with STAT1 alpha in wild-type Hela cells. Weakened bands were observed when STAT1 alpha knockdown samples were tested. Wild-type and STAT1 alpha knockdown samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1612-22, 1:500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG-HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

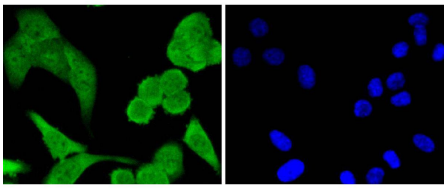

Fig3: ICC staining of STAT1 in SKOV-3 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1612-22, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

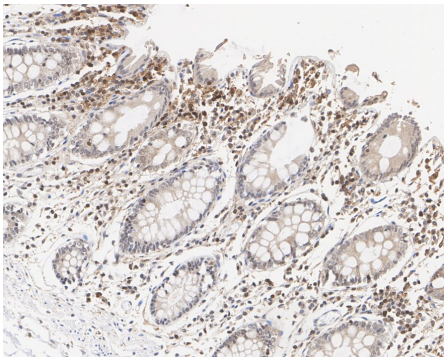

Fig4: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-STAT1 antibody (ET1612-22) at 1/500 dilution.

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 (ET1612-22) 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.

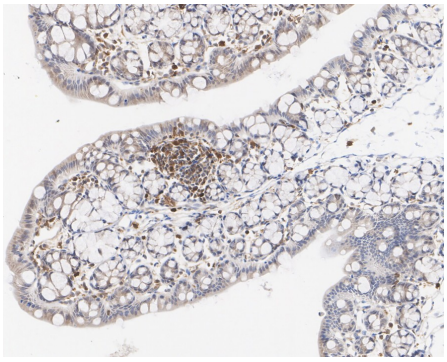

Fig5: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-STAT1 antibody (ET1612-22) at 1/2,000 dilution.

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 (ET1612-22) 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.

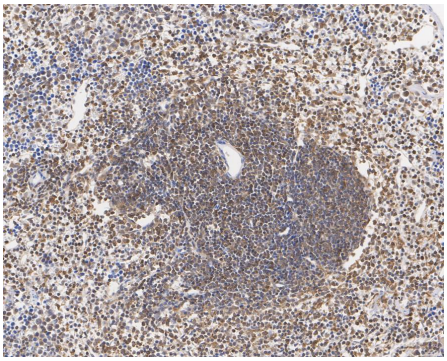

Fig6: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-STAT1 antibody (ET1612-22) at 1/2,000 dilution.

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 (ET1612-22) 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.

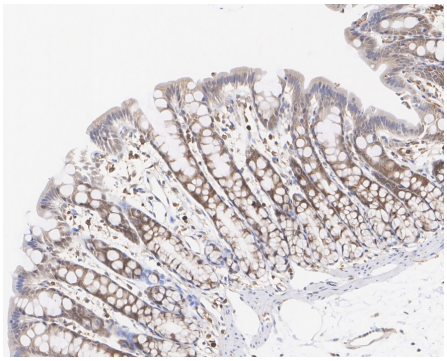

Fig7: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-STAT1 antibody (ET1612-22) at 1/2,000 dilution.

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 (ET1612-22) 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.

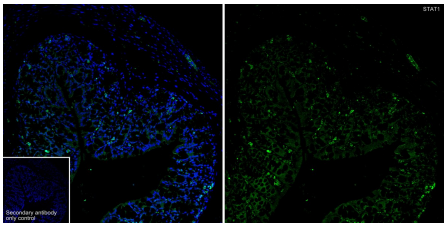

Fig8: Immunofluorescence analysis of paraffin-embedded mouse colon tissue labeling STAT1 with Rabbit anti-STAT1 antibody (ET1612-22) at 1/200 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1612-22, green) at 1/200 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

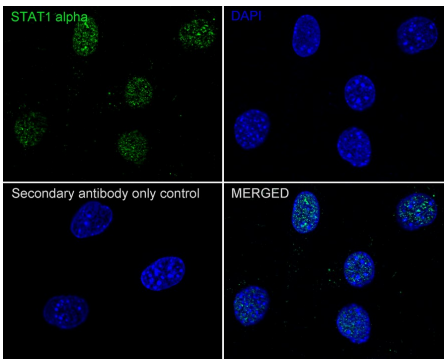

Fig9: Immunocytochemistry analysis of NIH/3T3 cells labeling STAT1 with Rabbit anti-STAT1 antibody (ET1612-22) at 1/100 dilution.

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 antibody (ET1612-22) 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.

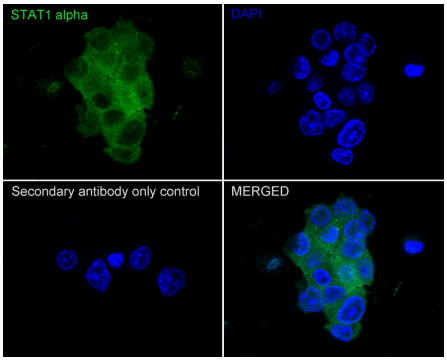

Fig10: Immunocytochemistry analysis of PC-12 cells labeling STAT1 with Rabbit anti-STAT1 antibody (ET1612-22) at 1/200 dilution.

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 antibody (ET1612-22) at 1/200 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.

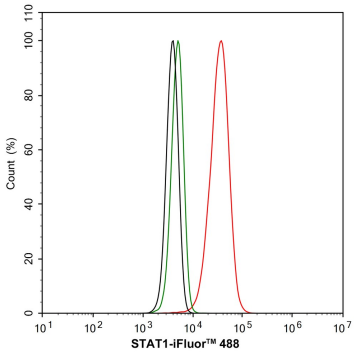

Fig11: Flow cytometric analysis of NIH/3T3 cells labeling STAT1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1612-22, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

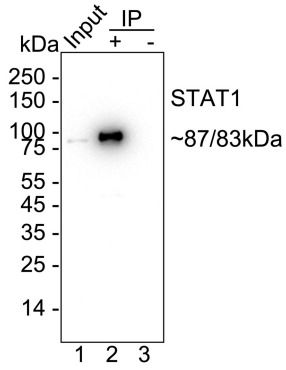

Fig12: STAT1 was immunoprecipitated from 0.2 mg HeLa cell lysate with ET1612-22 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1612-22 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: ET1612-22 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of ET1612-22 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 12 seconds; ECL: K1801

Background References

1. Nagakura I et al. STAT1 Regulates the Homeostatic Component of Visual Cortical Plasticity via an AMPA Receptor-Mediated Mechanism. J Neurosci 34:10256-63 (2014).
2. Carlos TS et al. Parainfluenza virus 5 genomes are located in viral cytoplasmic bodies whilst the virus dismantles the interferon-induced antiviral state of cells. J Gen Virol 90:2147-56 (2009).