Anti-Phospho-STAT1 (S727) Antibody [SN67-04] ET1611-20

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, FC
Molecular Wt:	Predicted band size: 87 kDa
Clone number:	SN67-04

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-a 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.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser727 of Human STAT1 aa 701-750 / 750.

Positive control: HeLa cell lysate, mouse brain tissue lysate, rat brain tissue lysate, HeLa, human breast invasive ductal tumor tissue, rat colon tissue, mouse colon tissue, SiHa cell lysates, human liver carcinoma tissue, human lung carcinoma tissue.

Subcellular location: Nucleus, Cytoplasm.

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

Recommended Dilutions:

WB	1:2,000-1:5,000
IHC-P	1:200-1:1,000
IF-Cell	1:2,000
FC	1:1,000

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

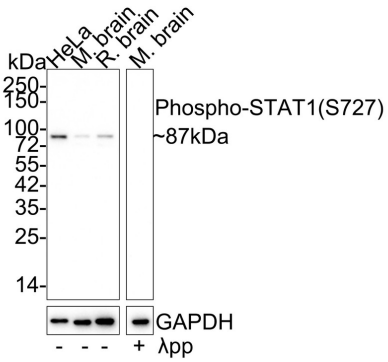

Fig1: Western blot analysis of Phospho-STAT1 (S727) on different lysates with Rabbit anti-Phospho-STAT1 (S727) antibody (ET1611-20) at 1/5,000 dilution.

Lane 1: HeLa cell lysate (15 µg/Lane)
Lane 2: Mouse brain tissue lysate (20 µg/Lane)
Lane 3: Rat brain tissue lysate (20 µg/Lane)
Lane 4: Mouse brain treated with λpp for 1 hour tissue lysate (20 µg/Lane)

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

Exposure time: 3 minutes 10 seconds;

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 (ET1611-20) at 1/5,000 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.

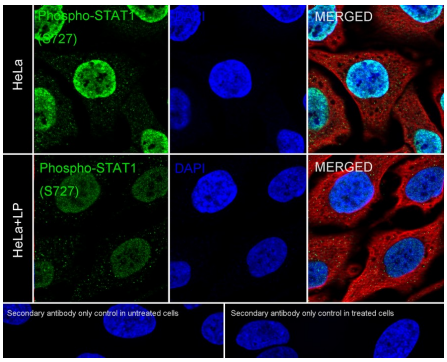

Fig2: Immunocytochemistry analysis of HeLa cells treated with or without λpp labeling Phospho-STAT1 (S727) with Rabbit anti-Phospho-STAT1 (S727) antibody (ET1611-20) at 1/2,000 dilution.

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-Phospho-STAT1 (S727) antibody (ET1611-20) at 1/2,000 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.

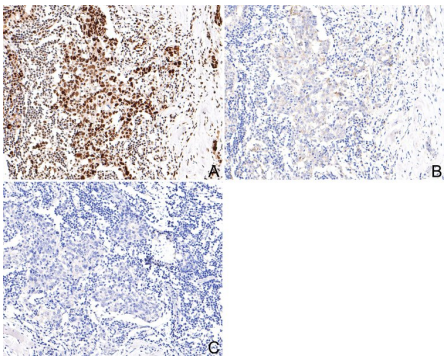

Fig3: Immunohistochemical analysis of paraffin-embedded human breast invasive ductal tumor tissue with Rabbit anti-Phospho-STAT1 (S727) antibody (ET1611-20) at 1/200 dilution.

A: Untreated human breast invasive ductal tumor tissue
B: λ-PPase treated human breast invasive ductal tumor tissue
C: Negative control

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 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 (ET1611-20) at 1/200 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.

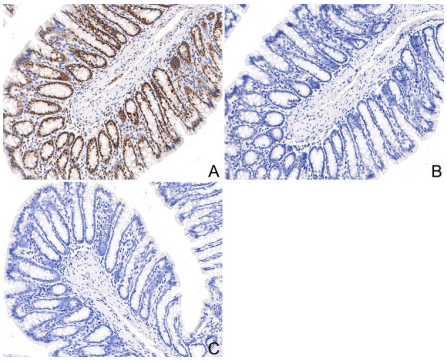

Fig4: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-Phospho-STAT1 (S727) antibody (ET1611-20) at 1/200 dilution.

A: Untreated rat colon tissue
B: λ-PPase treated rat colon tissue
C: Negative control

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 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 (ET1611-20) at 1/200 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.

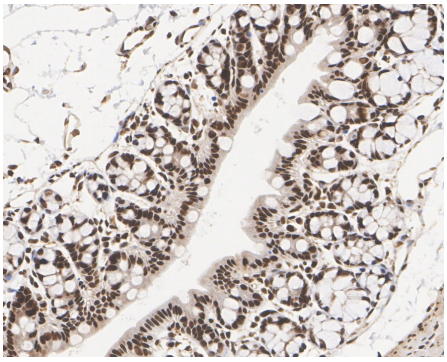

Fig5: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-Phospho-STAT1 (S727) antibody (ET1611-20) at 1/1,000 dilution.

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 (ET1611-20) at 1/1,000 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.

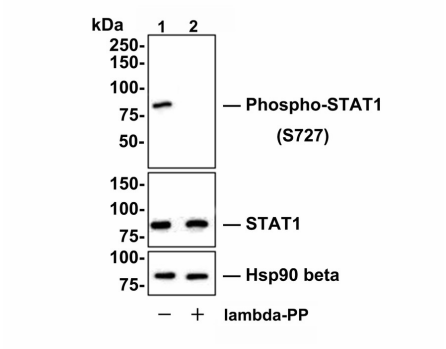

Fig6: Western blot analysis of Phospho-STAT1 (S727) on SiHa cell lysates.

Lane 1: SiHa cells, whole cell lysate, 10 µg /lane.
Lane 2: SiHa cells were treated with 2.8 µg/ul lambda-PP for 30 minutes, whole cell lysates, 10 µg/lane.

Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody Anti-Phospho-STAT1 (S727) (ET1611-20, 1/500) , Anti-STAT1 antibody (ET1606-39, 1/500) and Anti-HSP90 beta antibody (ET1605-56, 1/10,000)was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG H&L (HRP) Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

Predicted band size: 87 kDa
Observed band size: 87 kDa
Exposure time: 30 seconds

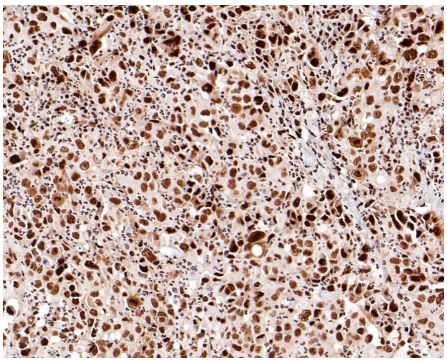

Fig7: Immunohistochemical analysis of paraffin-embedded human liver carcinoma tissue with Rabbit anti-Phospho-STAT1 (S727) antibody (ET1611-20) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 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 (ET1611-20) 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.

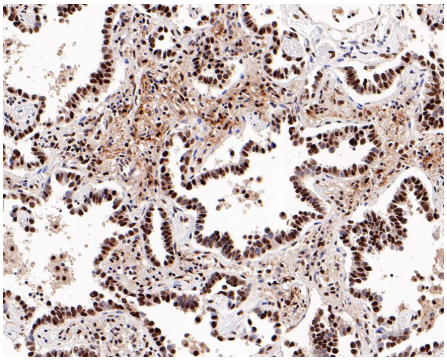

Fig8: Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue with Rabbit anti-Phospho-STAT1 (S727) antibody (ET1611-20) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 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 (ET1611-20) 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.

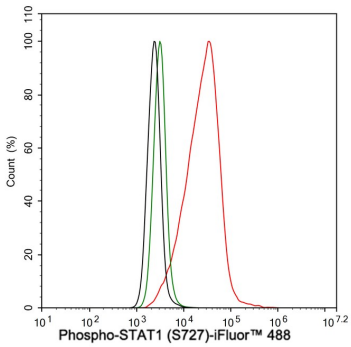

Fig9: Flow cytometric analysis of HeLa cells labeling Phospho-STAT1 (S727).

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1611-20, 1μg/mL) (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).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Chen L et al. Metastasis is regulated via microRNA-200/ZEB1 axis control of tumour cell PD-L1 expression and intratumoral immunosuppression. Nat Commun 5:5241 (2014).
2. Camicia R et al. BAL1/ARTD9 represses the anti-proliferative and pro-apoptotic IFN -STAT1-IRF1-p53 axis in diffuse large B-cell lymphoma. J Cell Sci 126:1969-80 (2013).