Anti-STAT3 Antibody [SY34-01] - BSA and Azide free

HA750087

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 88 kDa
Clone number:	SY34-01

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.

Immunogen: Synthetic peptide within N-terminal human STAT3.

Positive control: HeLa cell lysate, HaCaT cell lysate, Mouse brain tissue lysate, Rat brain tissue lysate, Rat kidney tissue lysate, HeLa, NIH/3T3, human stomach carcinoma tissue, human breast carcinoma tissue, mouse brain tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P40763 Human | P42227 Mouse | P52631 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100
IF-Tissue	1:100-1:500
IHC-P	1:50-1:1,000
FC	1:1,000

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

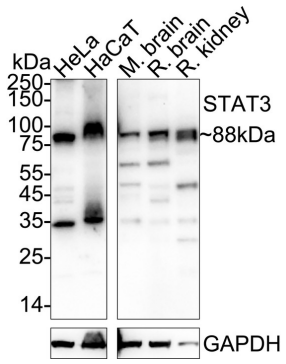

Fig1: Western blot analysis of STAT3 on different lysates with Rabbit anti-STAT3 antibody (HA750087) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HaCaT cell lysate
Lane 3: Mouse brain tissue lysate
Lane 4: Rat brain tissue lysate
Lane 5: Rat kidney tissue lysate

Lysates/proteins at 30 µg/Lane.

Predicted band size: 88 kDa
Observed band size: 88 kDa

Exposure time: 30 seconds; ECL: K1802;
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 (HA750087) at 1/2,000 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.

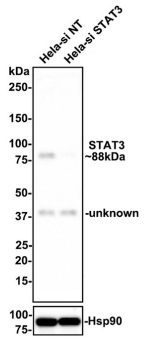

Fig2: Western blot analysis of STAT3 on different lysates with Rabbit anti-STAT3 antibody (HA750087) at 1/500 dilution.

Lane 1: Hela-si NT cell lysate
Lane 2: Hela-si STAT3 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 88 kDa
Observed band size: 88 kDa

Exposure time: 1 minute;
4-20% SDS-PAGE gel.

ET1605-45 was shown to specifically react with STAT3 in Hela-si NT cells. No band was observed when Hela-si STAT3 sample was tested. Hela-si NT and Hela-si STAT3 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 (ET1609-76, 1/500) and Loading control antibody (Rabbit anti-Hsp90, ET1605-56, 1/10,000) were 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.

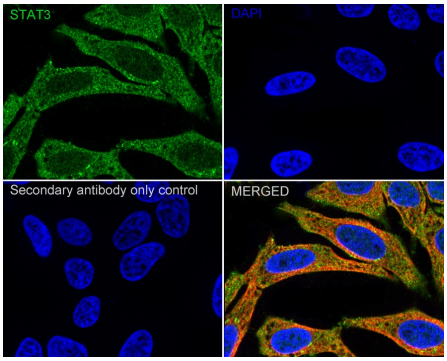

Fig3: Immunocytochemistry analysis of HeLa cells labeling STAT3 with Rabbit anti-STAT3 antibody (HA750087) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-STAT3 antibody (HA750087) 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 (HA601187, 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.

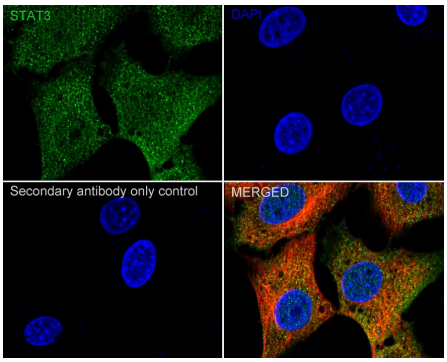

Fig4: Immunocytochemistry analysis of NIH/3T3 cells labeling STAT3 with Rabbit anti-STAT3 antibody (HA750087) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-STAT3 antibody (HA750087) 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 (HA601187, 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.

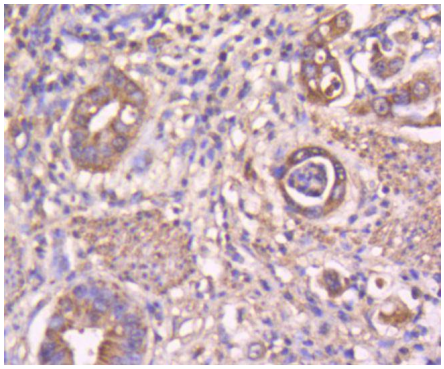

Fig5: Immunohistochemical analysis of paraffin-embedded human stomach carcinoma tissue using anti-STAT3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750087, 1/50) for 30 minutes 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.

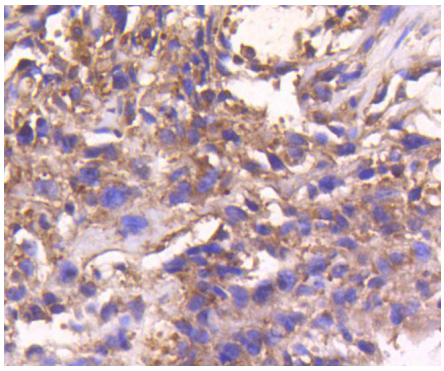

Fig6: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-STAT3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750087, 1/50) for 30 minutes 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.

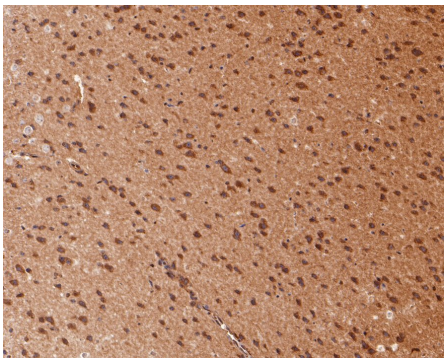

Fig7: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-STAT3 antibody (HA750087) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) 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 (HA750087) 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.

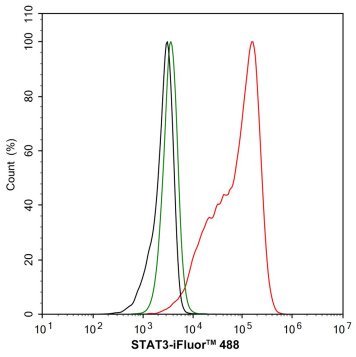

Fig8: Flow cytometric analysis of HeLa cells labeling STAT3.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750087, 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).

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

Background References

1. Fu TG et al. miR-143 inhibits oncogenic traits by degrading NUAK2 in glioblastoma. Int J Mol Med 37:1627-35 (2016).
2. Schwartz C et al. Melatonin receptor signaling contributes to neuroprotection upon arousal from torpor in thirteen-lined ground squirrels. Am J Physiol Regul Integr Comp Physiol 309:R1292-300 (2015).