Anti-Phospho-STAT5 (Y694) Antibody [SC05-31] - BSA and Azide free

HA750220

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Rat, Mouse
Applications:	WB, IHC-P, IF-Cell
Molecular Wt:	Predicted band size: 91 kDa
Clone number:	SC05-31

Description: Stat5 (Signal Transducers and Activators of Transcription 5) is important in regulating T cell functions involving the receptors for Interleukin-2 (IL-2). IL-2 stimulates the rapid phosphorylation of both serine and tyrosine residues of Stat5a and Stat5b in human T lymphocytes and in several IL-2-responsive lymphocytic cell lines. IL-2 differentially induces serine phosphorylation of Stat5a and Stat5b on Ser726 and Ser731, respectively. Stat5b is preferentially phosphorylated and displays more protracted serine phosphorylation kinetics than Stat5a. Both the acid-rich region and the COOH terminus of IL-2Rb can independently mediate IL-2-induced Stat 5a/b serine phosphorylation, suggesting that Stat5a/b serine phosphorylation occurs at a postreceptor level. Stat5a is phosphorylated on Tyr694 in a prolactin-sensitive manner, whereas serine phosphorylation is constitutive. Activation of Stat5 by IL-2 may help govern the biological effects of IL-2 during the immune response.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Tyr694 of human STAT5A.

Positive control: PC-12 treated with 1mM Sodium orthovanadate for 30 minutes cell lysate, PC-12 cells treated with 1mM Sodium orthovanadate for 30 minutes, Hela cells treated with 100ng/mL IFN alpha for 60 minutes, human breast carcinoma tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P42229 Human | P51692 Human | P42230 Mouse | P42232 Mouse | P52632 Rat | Q62771 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IHC-P	1:30,000
IF-Cell	1:100

Storage Buffer: 1*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

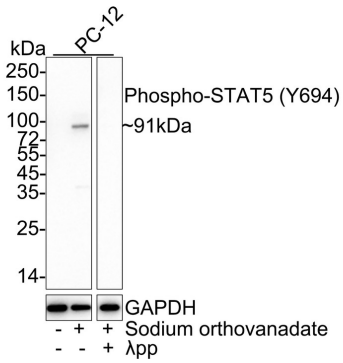

Fig1: Western blot analysis of Phospho-STAT5 (Y694) on different lysates with Rabbit anti-Phospho-STAT5 (Y694) antibody (HA750220) at 1/1,000 dilution.

Lane 1: PC-12 cell lysate
Lane 2: PC-12 treated with 1mM Sodium orthovanadate for 30 minutes cell lysate
Lane 3: PC-12 treated with 1mM Sodium orthovanadate for 30 minutes cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 20 µg/Lane.

Predicted band size: 91 kDa
Observed band size: 91 kDa

Exposure time: 3 minutes;
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 (HA750220) 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.

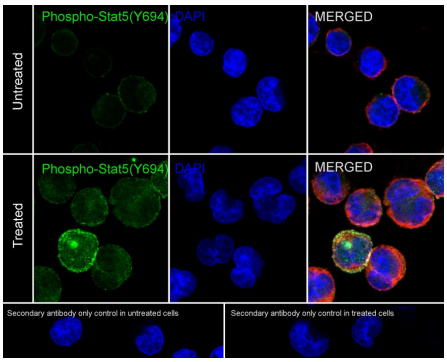

Fig2: Immunocytochemistry analysis of PC-12 cells treated with 1mM Sodium orthovanadate for 30 minutes labeling Phospho-STAT5 (Y694) with Rabbit anti-Phospho-STAT5 (Y694) antibody (HA750220) at 1/100 dilution.

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-Phospho-STAT5 (Y694) antibody (HA750220) 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.

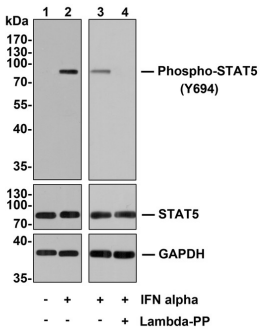

Fig3: Western blot analysis of Phospho-STAT5(Y694) on Hela cell lysates.

Lane 1: Hela cells, whole cell lysate, 10ug/lane
Lane 2/3: Hela cells treated with 100ng/mL IFN alpha for 60 minutes, whole cell lysates, 10ug/lane
Lane 4: Hela cells treated with 100ng/mL IFN alpha for 60 minutes, then treated with 2.8ug/ul lambda-PP for 30 minutes, whole cell lysates, 10ug/lane

All lanes :
Anti-Phospho-STAT5(Y694) antibody (HA750220) at 1:500 dilution. Anti-STAT5 antibody (ET1612-63) at 1:500 dilution. Anti-GAPDH antibody (ET1601-4) at 1:10,000 dilution. Goat Anti-Rabbit IgG H&L (HRP) (HA1001) at 1/200,000 dilution.

Predicted band size: 91 kDa
Observed band size: 91 kDa

Blocking and diluting buffer: 5% BSA.
Exposure time: 2 minutes 2 seconds

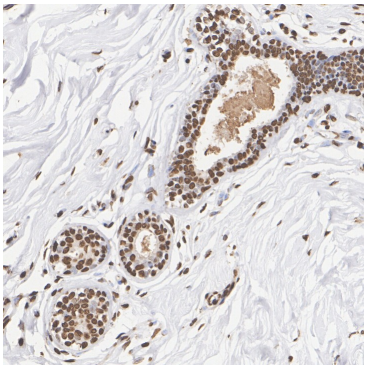

Fig4: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-Phospho-STAT5 (Y694) antibody (HA750220) at 1/30,000 dilution.

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 (HA750220) at 1/30,000 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.

Background References

1. Yang TY et al. A multiple reaction monitoring (MRM) method to detect Bcr-Abl kinase activity in CML using a peptide biosensor. PLoS One 8:e56627 (2013).
2. Caldarelli A et al. A genome-wide RNAi screen identifies proteins modulating aberrant FLT3-ITD signaling. Leukemia 27:2301-10 (2013).