Anti-STAT5b Antibody [SD08-08]

ET1612-63

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 90 kDa
Clone number:	SD08-08

Description: Signal transducer and activator of transcription 5A (Stat5a) and Stat5b, which share 96% homology, undergo receptor tyrosine kinase or G protein-coupled receptor-dependent phosphorylation in response to cytokines or growth factors, and then form homo- or heterodimers that translocate to the nucleus, where they initiate transcription. Activation of Stat5a via IL-2, IL-3, IL-7/ GM-CSF, erythropoietin, thrombopoietin and growth hormones influences proliferation, differentiation and apoptosis in lymphohematopoietic cells. Phosphorylation of Stat5a at Ser127/Ser128 and Ser779 are contigent on ErbB-4-mediated activation of Stat5a. Activation of Stat5b via IL-2, IL-4, CSF-1 and growth hormones influences TCR signaling, apoptosis, adult mammary gland development and sexual dimorphism of liver gene expression. Stat5b is the major liver-expressed Stat5 form that has been shown to fuse with the retin-oic acid receptor a gene in acute promyelocytic leukemias (APLL). Stat5a/b null mice have severely impaired lymphoid development and differentiation.

Immunogen: Recombinant protein within C-terminal mouse STAT5b.

Positive control: A549-si NT cell lysate, A549-si 商品名 cell lysate, A549 cell lysate, HeLa cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, Mouse brain tissue lysate, Mouse testis tissue lysate, Rat ovary tissue lysate, A549, NIH/3T3, PC-12, human breast carcinoma tissue, mouse colon tissue, human spleen tissue, mouse testis tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P51692 Human | P42232 Mouse | P52632 Rat

Recommended Dilutions:

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

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

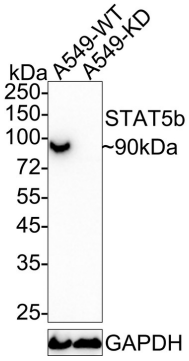

Fig1: Western blot analysis of STAT5b on different lysates with Rabbit anti-STAT5b antibody (ET1612-63) at 1/1,000 dilution.

Lane 1: A549-si NT cell lysate
Lane 2: A549-si STAT5b cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 90 kDa
Observed band size: 90 kDa

Exposure time: 30 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 (ET1612-63) 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.

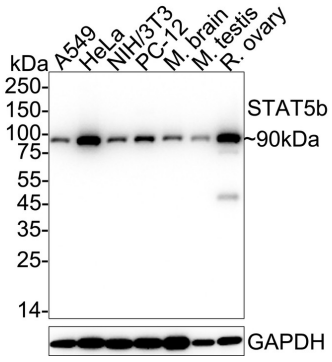

Fig2: Western blot analysis of STAT5b on different lysates with Rabbit anti-STAT5b antibody (ET1612-63) at 1/1,000 dilution.

Lane 1: A549 cell lysate (10 µg/Lane)
Lane 2: HeLa cell lysate (10 µg/Lane)
Lane 3: NIH/3T3 cell lysate (10 µg/Lane)
Lane 4: PC-12 cell lysate (10 µg/Lane)
Lane 5: Mouse brain tissue lysate (20 µg/Lane)
Lane 6: Mouse testis tissue lysate (20 µg/Lane)
Lane 7: Rat ovary tissue lysate (20 µg/Lane)

Predicted band size: 90 kDa
Observed band size: 90 kDa

Exposure time: 4 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 (ET1612-63) 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.

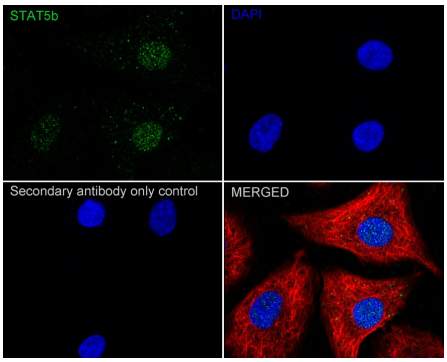

Fig3: Immunocytochemistry analysis of A549 cells labeling STAT5b with Rabbit anti-STAT5b antibody (ET1612-63) at 1/50 dilution.

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-STAT5b antibody (ET1612-63) at 1/50 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.

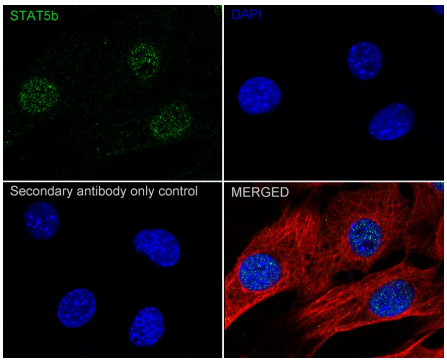

Fig4: Immunocytochemistry analysis of NIH/3T3 cells labeling STAT5b with Rabbit anti-STAT5b antibody (ET1612-63) at 1/50 dilution.

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-STAT5b antibody (ET1612-63) at 1/50 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.

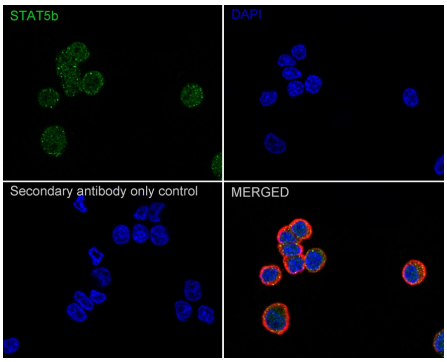

Fig5: Immunocytochemistry analysis of PC-12 cells labeling STAT5b with Rabbit anti-STAT5b antibody (ET1612-63) at 1/50 dilution.

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-STAT5b antibody (ET1612-63) at 1/50 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.

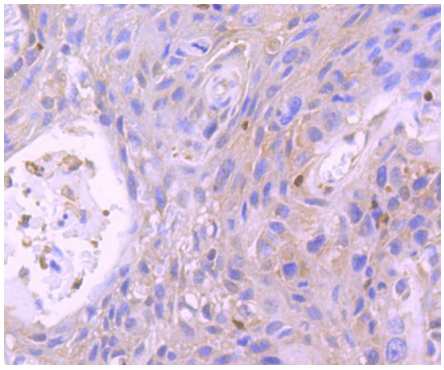

Fig6: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-STAT5b antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1612-63, 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.

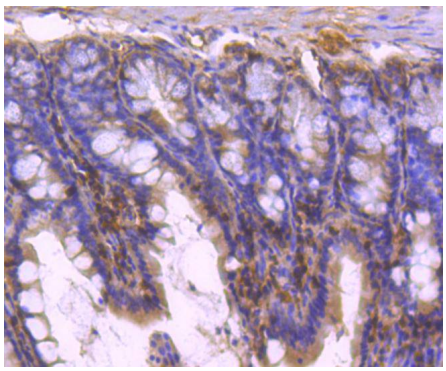

Fig7: Immunohistochemical analysis of paraffin-embedded mouse colon tissue using anti-STAT5b antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1612-63, 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.

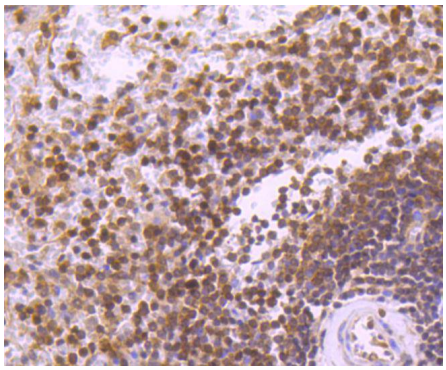

Fig8: Immunohistochemical analysis of paraffin-embedded human spleen tissue using anti-STAT5b antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1612-63, 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.

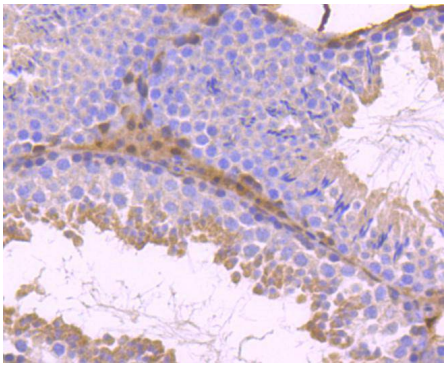

Fig9: Immunohistochemical analysis of paraffin-embedded mouse testis tissue using anti-STAT5b antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1612-63, 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.

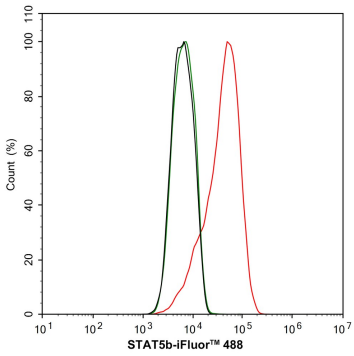

Fig10: Flow cytometric analysis of PC-12 cells labeling STAT5b.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1612-63, 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).

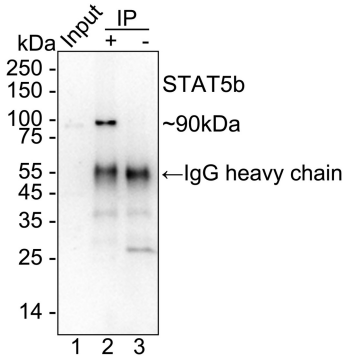

Fig11: STAT5b was immunoprecipitated from 0.2 mg HeLa cell lysate with ET1612-63 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using ET1612-63 at 1/2,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: ET1612-63 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of ET1612-63 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 1 minute; ECL: K1802

Background References

1. Diamantopoulos, PT. et al. 2016. Prognostic significance of signal transducer and activator of transcription 5 and 5b expression in Epstein-Barr virus-positive patients with chronic lymphocytic leukemia. Cancer Med. 5: 2240-8.
2. Prost, S. et al. 2015. Erosion of the chronic myeloid leukaemia stem cell pool by PPARγ agonists. Nature. 525: 380-3.