

Affinity-Purified Rabbit Anti-human/mouse STAT5b Antibody

ORDERING INFORMATION

Catalog Number: AF1584

Lot Number: JFQ01

Size: 50 µg (sufficient for 500 mL of blotting solution)

Storage: -20° C

Specificity: human and mouse STAT5b

Immunogen: synthetic peptide corresponding to aa 777 - 787

Ig Type: rabbit IgG

Applications: Western blot
Immunoprecipitation
Flow Cytometry

Preparation

Rabbit antibodies were raised against a synthetic peptide corresponding to amino acids 777 - 787 of human STAT5b. Polyclonal antibody was affinity-purified on a column derivatized with the peptide, dialyzed and concentrated.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute in 50 µL of PBS containing 0.02% NaN₃.

Storage

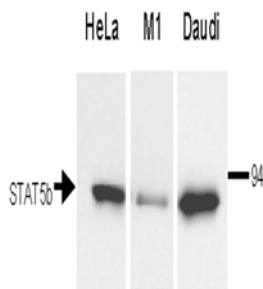
Lyophilized antibody is stable for twelve months from date of receipt when stored at -20° C to -70° C. Avoid repeated freezing and thawing by aliquoting smaller portions of reconstituted antibody into Eppendorf tubes and storing at -20° to -70°C in a manual defrost freezer.

Specificity

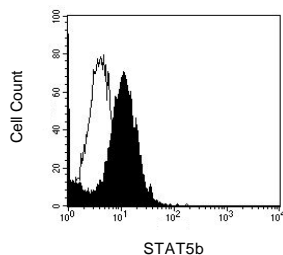
The antibody detects endogenous human and mouse STAT5b.

Applications

Western blot - An antibody concentration of 0.1 µg/mL is recommended.



Detection of STAT5B with AF1584. Lysates from 5×10^5 human HeLa and Daudi cells and mouse M1 cells were resolved by SDS-PAGE, and immunoblotted with 0.1 µg/mL rabbit anti-STAT5b as described.



Human Jurkat cells were stained with anti-STAT5b (R&D Systems, Catalog # AF1584, filled histogram) or isotype control (R&D Systems, Catalog # AB-105-C, open histogram), followed by APC-conjugated anti-rabbit antibody.

Protocols for Western Blotting

Blotting Buffer	Blocking Solution	Antibody solution
25 mM Tris, pH 7.5	5% nonfat dry milk	5% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.01% Tween 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to Immobilon membrane (Millipore) and incubate the membrane for 1 hour at room temperature in blocking Solution.
2. Incubate the membrane overnight at 4° C in Antibody Solution containing 0.5 µg/mL rabbit anti-human/mouse STAT5b.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated goat anti-rabbit IgG (R&D Systems, Catalog # HAF008).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with ECL Reagent (Amersham).

Cell lysates for Western blottings - To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF and bromophenyl blue) at 2×10^6 - 1×10^7 cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each. Samples are diluted with 1x SDS sample buffer to the desired concentration.

Flow Cytometry - For intracellular staining to detect STAT5b, cells must first be fixed and permeabilized using 2% paraformaldehyde and ice-cold methanol. Dilute this antibody to 25 µg/mL and add 10 µL of the diluted solution to $1-5 \times 10^5$ cells in a total reaction volume not exceeding 200 µL. Following a 30 minute incubation, cells should be washed in an isotonic phosphate buffer (supplemented with 0.5% BSA) prior to the addition of a secondary developing reagent. The binding of unlabeled polyclonal antibodies may be visualized by adding 10 µL of a 25 µg/mL solution of a secondary developing reagent such as anti-rabbit IgG conjugated to a fluorochrome. Cells should be washed for a final time prior to flow cytometric analysis.

Immunoprecipitation - 2 µg rabbit anti-STAT5b is sufficient to perform immunoprecipitation of 5×10^6 cells.

Protocols for Immunoprecipitation with anti-STAT1 p91:

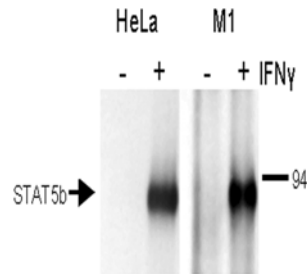
Nuclear Extracts for immunoprecipitations - Wash cells with PBS, making sure to remove any remaining PBS after centrifugation. Solubilize cells at 2.5×10^6 cells/mL in Lysis Buffer A (10 mM Hepes pH 7.9, 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM DTT, 0.1% NP-40, 2 mM Na₃VO₄, 25 µg/mL Leupeptin, 25 µg/mL Pepstatin, 25 µg/mL Chymostatin, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), 3 µg/mL Aprotinin) by pipetting up and down. Centrifuge at 16,000 x g for 5 minutes at 4°C in a microcentrifuge. Remove cytosolic supernatant and solubilize nuclear pellet in Lysis Buffer B (20 mM Hepes pH 7.9, 1.5 mM MgCl₂, 420 mM NaCl, 0.5 mM DTT, 25% glycerol, 2 mM Na₃VO₄, 25 µg/mL Leupeptin, 25 µg/mL Pepstatin, 25 µg/mL Chymostatin, 0.2 mM PMSF, 3 µg/mL Aprotinin) at 1×10^8 cells/mL. Vortex 10 seconds and incubate on ice for 20 minutes. Centrifuge at 16,000 x g for 5 minutes at 4° C. Transfer supernatant to a fresh ice-cold tube, aliquot, and store at -70° C.

Whole cell extracts for immunoprecipitations:

Cells grown in suspension are rinsed three times with phosphate buffered saline by centrifugation. Cell protein is extracted by solubilizing the cell pellet at 1×10^6 - 5×10^6 cells per mL in cold extraction buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 0.02% NaN₃, 10 mM NaF, 1 mM sodium ortho-vanadate, 0.25 mM phenylmethylsulfonyl fluoride (PMSF), 1 µg/mL aprotinin, 1 µg/mL leupeptin, and 1 µg/mL chymostatin). Cells grown in monolayers are rinsed three times with phosphate buffered saline and then solubilized by scraping into 2 - 3 mL of cold extraction buffer. The extraction mixtures are rocked at 2° - 8° C for 30 - 60 min. The lysates are then centrifuged at 3,000 x g for 5 min. to remove insoluble material.

Immunoprecipitation:

Rabbit anti-STAT5b is added to 50 µL extracts and 50 µL 2X IP buffer (20 mM Tris, pH 7.4, 300 mM NaCl, 2 mM EDTA, 2 mM EGTA, 1% NP-40, 2% Triton X-100, 0.4 mM sodium orthovanadate) and the mixture is rocked for 1 hour at room temperature. Protein A sepharose (Zymed, Catalog # 10-1041) is then added and the mixture is rocked for an additional 1 hour. The Protein A- absorbed complexes are centrifuged for 0.5 min. in an Eppendorf centrifuge, resuspended in 1X IP buffer, and then repelleted. The complexes are washed a total of four times then suspended in 50 µL of 2 x SDS gel sample buffer (0.25 M Tris, pH 6.8, 6% SDS, 10% Glycerol, 20 mM Dithiothreitol and bromophenyl blue) by vortexing and then incubated for 3 min. in a boiling water bath. Protein A is pelleted and the supernatant is loaded on a polyacrylamide gel.



Immunoprecipitation of STAT5b. Cell extracts from human HeLa and mouse M1 cells activated with IFN- γ were incubated with Rabbit anti-STAT5b and protein A. Immunoprecipitates were electrophoresed on a 5 - 15% SDS polyacrylamide gel and transferred to Immobilon. STAT5b was detected by immunoblotting with anti-phospho-STAT5 antibody.

Optimal dilutions should be determined by each laboratory for each application.