Anti-STAT 5A Serum

ORDERING INFORMATION

Catalog Number: PA-ST5A

Lot Number: RU07

Size: 200 µL

Storage: -20° C

Specificity: STAT 5A

Immunogen: aa 775 - 794 of murine STAT 5A

Host: Rabbit

Applications: Western blot
Immunoprecipitation

Preparation
Rabbits were immunized with the synthetic peptide LDARLSPPAGLFTSARSSLS which corresponds to amino acids 775 - 794 of murine STAT 5A.

Formulation
Lyophilized from 0.2 mL antiserum containing 0.02% sodium azide.

Reconstitution
Dissolve in 0.2 mL water.

Storage
Avoid repeated freezing and thawing by aliquoting smaller portions of the reconstituted serum into Eppendorf tubes and storing at -20° C or -70° C **in a manual defrost freezer.**

Specificity
The anti-serum is known to react with human and murine STAT 5A and does not cross-react with any of the other Stat proteins.

Western blot
A 1:2,000 dilution of the reconstituted anti-serum is recommended.

Immunoprecipitation
3 µL per immunoprecipitation of STAT 5A from 10^6 - 10^7 cells is recommended.

The amino acid sequence of the peptide used to generate anti-STAT 5A is not found in STAT 5B and anti-STAT 5A does not react with STAT 5B. The amino acid sequence of the peptide used to generate anti-STAT 5B has a 5 amino acid sequence that is also found in STAT 5A and anti-STAT 5B has partial reactivity with STAT 5A.

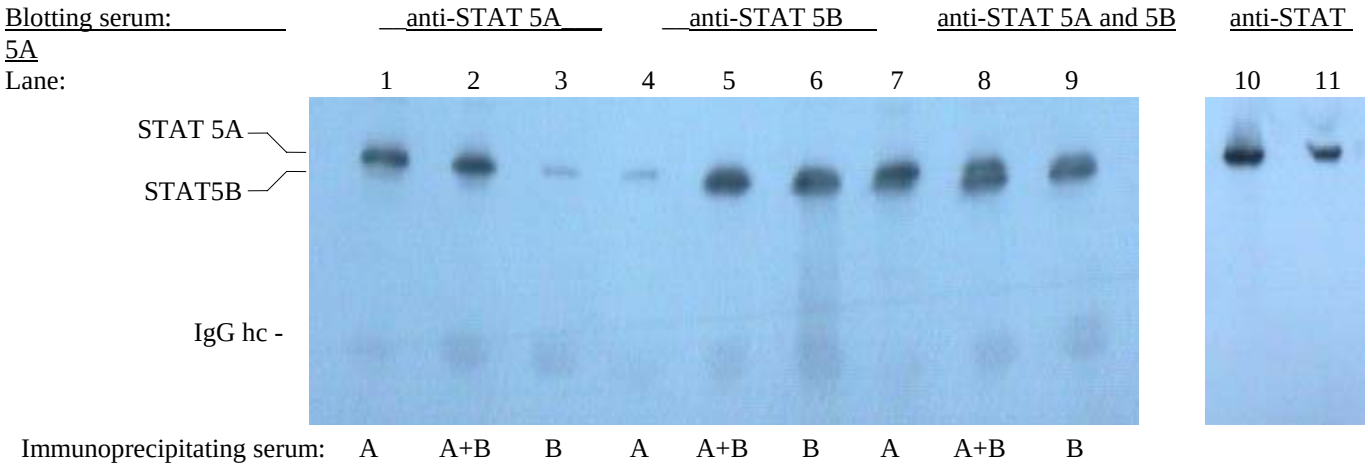

Figure:
Western blots of STAT 5A and STAT 5B immunoprecipitated with 3 µL antiserum/immunoprecipitation from 5 x 10^6 CTLL2 cells. Western transfers were blotted with anti-STAT 5A (lanes 1 - 3), anti-STAT 5B (lanes 4 - 6), or both antisera (lanes 7 - 9). Immunoprecipitations were with anti-STAT 5A (lanes 1, 4, and 7), anti-STAT 5B (lanes 3, 6, and 9) or both (lanes 2, 5, and 8). Anti-STAT 5A does not immunoprecipitate or blot STAT 5B. Using these conditions, the amount of STAT 5A is less than 5% of the amount of STAT 5B in immunoprecipitations using anti-STAT 5B (compare lane 3 to lane 6). Western blotting with anti-STAT 5B of anti-STAT 5A immunoprecipitated protein (lane 4) also showed a low level of detection compared to detection with anti-STAT 5A (lane 1). Lanes 10 and 11 are the respective immunoblots of STAT 5A in total cell lysates from 1 x 10^6 and 5 x 10^5 CTLL2 cells.

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Protocols for Immunoprecipitation and Immunoblotting with anti-STAT 5A:

Cell lysis 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 x 10^6 - 1 x 10^7 cells per ml of cold extraction buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 0.02% NaN_3, 10 mM NaF, 1 mM sodium ortho-vanadate, 0.25 mM phenylmethylsulfonyl fluoride, 1 μg/mL aprotinin, 1 μg/mL leupeptin, and 1 μg/mL chymostatin) and rocking the mixture a 2° - 8° C for 30 - 60 minutes. The lysate is then centrifuged at 12,000 x g for 5 minutes to remove insoluble material. One mL of cell lysate is precleared by incubation with 10 μL of a 20% suspension of fixed Staph A cells (Immunoprecipitin, BRL/Gibco) for 5 minutes on ice. Staph A cells are pelleted by centrifugation at 12,000 x g for 0.5 minute in an Eppendorf centrifuge, the supernatant is transferred to a new tube, and the preclearing is repeated one or more times. The Staph A cells had been washed three times with extraction buffer before being added to the extracts.

Immunoprecipitation:
Rabbit anti-STAT 5A is added to the 1 mL extract and the mixture is rocked for 1 hour at 2° - 8° C. Staph A cells (25 μL of a 20% suspension) are then added and the mixture is rocked in the cold for another hour. The staph A-absorbed complexes are centrifuged for 0.5 minutes in an Eppendorf centrifuge, resuspended in extraction buffer by trituration with a glass Pasteur pipet, and then repelleted. The complexes are washed a total of four times with extraction buffer, and then suspended in phosphate buffered saline and transferred to a new tube before the final centrifugation. The washed pellet is suspended in 25 μL of 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, and bromophenyl blue) by vortexing and then incubated for 3 minutes in a boiling water bath. Staph A cells are pelleted and the supernatant is loaded on a polyacrylamide gel.

Total cell lysates for immunoblotting:
The cell pellet is solubilized in hot 2x SDS gel sample buffer at 1 x 10^7 - 1 x 10^8 cells per mL and heated in a boiling water bath for 5 minutes. When the material is too viscous to pipet, it is sonicated for 10 - 20 seconds. Samples are diluted with 1x SDS sample buffer to the desired concentration.

Western blotting:
Proteins, electrophoresed on 14 cm x 9 cm x 1.5 mm, 5-15% polyacrylamide gradient gels, are transferred to Immobilon filters (Millipore) at room temperature at 250 mA per slab for 1 hour on a MilliBlot-Graphite Electroblotter II (Millipore). After blocking with 3% BSA in TBS (50 mM Tris, pH 7.4, 0.5 M NaCl, 0.05% Tween 20) for 1 hour at room temperature, the membrane is incubated overnight at 2° - 8° C in TBS containing 1% BSA and a 1:2,000 dilution of rabbit anti-STAT 5A serum. The membrane is washed for 1 hour with 5 or more changes of TBS and then incubated with TBS containing 1% BSA and a 1:1,000 dilution of HRP-conjugated Protein A (Amersham) for 1 hour at room temperature. The filter is washed as described for the primary antibody and immunodetected bands are visualized with the ECL system of Amersham.