Anti-STAT 6 Serum

ORDERING INFORMATION

Catalog Number: PA-ST6

Lot Number: RW07

Size: 200 µL

Storage: -20° C

Specificity: STAT 6

Immunogen: aa 818 - 837 of human STAT 6

Host: Rabbit

Applications: Western blot
Immunoprecipitation

Preparation

Rabbits were immunized with the synthetic peptide HYGQSGISMSHMDLRANPSW which corresponds to amino acids 818 - 837 of human STAT 6.

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 antiserum is known to react with human and murine STAT 6. This antiserum does not crossreact with any of the other Stat proteins.

Western blot

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

Immunoprecipitation

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

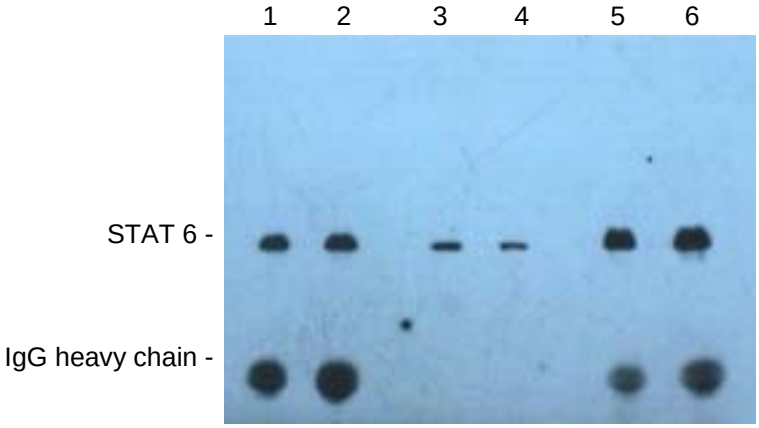

Figure:
Western blot of STAT 6 immunoprecipitated from 1 x 10^6 TF1 cells (lane 1 and 2) and from 2 x 10^6 human peripheral blood lymphocytes (lanes 5 and 6). Immunoprecipitation was with 3 µL (lanes 1 and 5) or 6 µL (lanes 2 and 6) of antiserum. Lanes 3 and 4 are the respective immunoblots of the total cell lysate from 6 x 10^5 and 3 x 10^5 TF1 cells. Proteins were electrophoresed on 5 - 15% polyacrylamide gels and immunoreactive bands were detected by the ECL system (Amersham) procedure. All procedures are described on the following page.

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Protocols for Immunoprecipitation and Immunoblotting with anti-STAT 6:

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 a 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 6 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 6 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.