

Anti-phospho-STAT5A/B (S726/731)

(rabbit antiserum)

Catalog # 06-867

Lot # 17444

Immunogen: KLH-conjugated, synthetic peptide (DQAP[pS]PAVC) corresponding to amino acids 722-730 of human STAT5A or 727-735 of human STAT5B.

Specificity: This antibody specifically recognizes phosphorylated STAT5A (S726) and STAT5B (S731). Does not recognize STAT5B containing a S731A mutation in lysates of transfected Cos-1 cells, stimulated with prolactin, as demonstrated by an independent laboratory.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

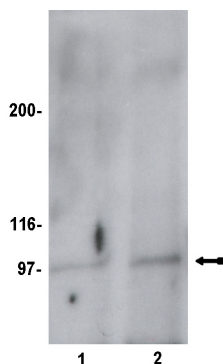
Formulation: 200µl of rabbit antiserum in 30% glycerol and 0.1% sodium azide. Liquid at -20°C.

Species Cross-reactivity: Human, mouse and rat; predicted cross-reactivity with bovine and sheep.

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoprecipitation/Immunoblot Analysis: 1:1000 dilution of this lot detected phosphorylated STAT5A following immunoprecipitation of STAT5A from RIPA lysates of Nb2-Sp cells that had been treated with prolactin; 4µl of anti-STAT5A (Catalog # 06-968) was used to immunoprecipitate total STAT5A.



Immunoprecipitation/Immunoblot Analysis:

Rat Nb2-Sp T lymphoma cell lysates were prepared from cells that were treated without (lane 1) or with (lane 2) prolactin for 15 minutes. STAT5A was immunoprecipitated with 4µl of anti-STAT5A. The immunoprecipitate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-STAT5A/B (S726/731) at 1:1000. Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phosphorylated STAT5A (~95kDa).

Background: Many hormones and cytokines selectively activate transcription factors of the STAT gene family. STAT5 was originally cloned as a prolactin-stimulated DNA-binding protein, but is also activated by non-lactogenic cytokines in many cell types.¹ Phosphorylation of a conserved tyrosine residue is required for STAT dimerization, nuclear translocation, and specific DNA binding. Serine residues located in a conserved PSP motif of STAT5A (S726) and STAT5B (S731) are also major phosphorylation sites. Interestingly, control of the phosphorylation of the PSP motif differs between the STAT5A (S725) and STAT5B (S730) serine sites.²

Application References:

1. Yamashita H., *et al.*, *J. Biol. Chem.* **273**: 30218-30224, 1998.
2. Kirken R.A., *et al.*, *J. Biol. Chem.* **272**: 14098-14103, 1997.
3. Kirken R.A., *et al.*, *J. Biol. Chem.* **272**: 15459-15465, 1997.

Immunoprecipitation/Immunoblot Protocol

1. Prepare cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1µg/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 1mM NaF; 1mM Microcystin).
2. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1µg/µl total cell protein in a microcentrifuge tube with PBS.
3. Add **4µl** of **a- STAT5A** (Catalog # 06-968) to 500µg-1mg cell lysate.
4. Gently rock the reaction mixture at 4°C overnight.
5. Capture the immunocomplex by adding 100µl of washed Protein A agarose bead slurry (50µl packed beads).
6. Gently rock the reaction mixture at 4°C for 2 hours.
7. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
8. Resuspend the agarose beads in 60µl 2X Laemmli sample buffer.
9. Collect the beads by a microcentrifuge pulse. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a sample of the supernatant and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
10. Block the blotted nitrocellulose in freshly prepared TBS containing 5% nonfat dry milk with 0.01% Tween-20 (TBST-MLK) for 20 minutes at 20-25°C with constant agitation.
11. Incubate the nitrocellulose with **1:1000 of a-phospho-Stat5A/B (S726/731)**, diluted in freshly prepared TBST-MLK overnight with agitation at 4°C.
12. Wash the nitrocellulose twice with water.
13. Incubate the nitrocellulose in the secondary reagent of choice (a **goat a-rabbit** HRP conjugated IgG, 1:3000 dilution was used) in TBST-MLK for 1.5 hours at room temperature with agitation.
14. Wash the nitrocellulose with water twice.
15. Wash the nitrocellulose in TBS-0.05% Tween 20 for 3-5 minutes.
16. Soak the nitrocellulose in water for 10 minutes, 4-5 times.
17. Use detection method of choice (enhanced chemiluminescence was used).