

#9351 Store at -20°C

Phospho-Stat5 (Tyr694) Antibody

- Small 100 μ l (10 western blots)
- Large 300 μ l (30 western blots)



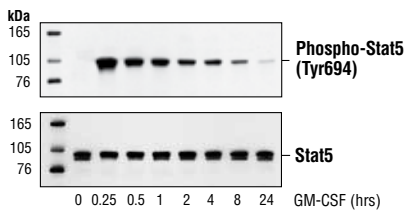
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rev. 11/11/11

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, F, ChIP Endogenous	H, M, (R, B)	90 kDa	Rabbit**

Background: Stat5 is activated in response to a wide variety of ligands including IL-2, GM-CSF, growth hormone and prolactin. Phosphorylation at Tyr694 is obligatory for Stat5 activation (1,2). This phosphorylation is mediated by Src upon erythropoietin stimulation (3). Stat5 is constitutively active in some leukemic cell types (4). Phosphorylated Stat5 is found in some endothelial cells treated with IL-3, which suggests its involvement in angiogenesis and cell motility (5). Stat5a and Stat5b are independently regulated and activated in various cell types. For instance, interferon treatment predominantly activates Stat5a in U-937 cells and Stat5b in HeLa cells (6).



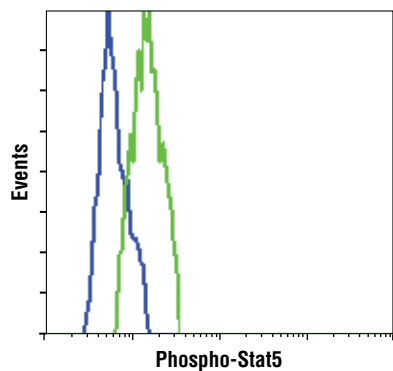
Western blot analysis of extracts from TF-1 cells, treated with GM-CSF (25 ng/ml) for the indicated times, using Phospho-Stat5 (Tyr694) Antibody (upper) or Stat5 antibody (lower).

Specificity/Sensitivity: Phospho-Stat5 (Tyr694) Antibody detects endogenous levels of Stat5a only when phosphorylated at Tyr694 and Stat5b when phosphorylated at Tyr699. The antibody does not cross-react with the corresponding phospho-tyrosine residues of other Stat proteins, but does cross-react with EGFR.

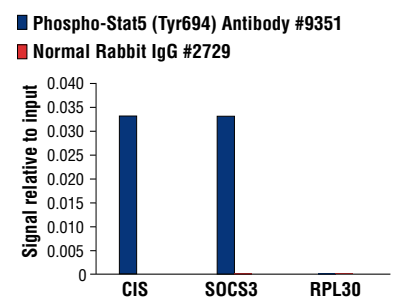
Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Tyr694 of mouse Stat5a. Antibodies are purified by protein A and peptide affinity chromatography.

Background References:

- Gouilleux, F. et al. (1994) *EMBO J.* 13, 4361-4369.
- Wakao, H. et al. (1994) *EMBO J.* 13, 2182-2191.
- Okutani, Y. et al. (2001) *Oncogene* 20, 6643-6650.
- Demoulin, J.B. et al. (1999) *J. Biol. Chem.* 274, 25855-25861.
- Dentelli, P. et al. (1999) *J. Immunol.* 163, 2151-2159.
- Meinke, A. et al. (1996) *Mol. Cell. Biol.* 16, 6937-6944.



Flow cytometric analysis of K562 cells, untreated (green) or STI571-treated (blue), using Phospho-Stat5 (Tyr694) Antibody.



◀ **Chromatin immunoprecipitations were performed with cross-linked chromatin from 4×10^6 BaF3 cells starved of IL-3 for 6 hours followed by induction with IL-3 for 45 minutes, and either 20 μ l of Phospho-Stat5 (Tyr694) Antibody or 2 μ l of Normal Rabbit IgG #2729 using SimpleChIP™ Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by Real-Time PCR using SimpleChIP™ Mouse CIS Intron 1 Primers #5131, mouse SOCS3 promoter primers, and SimpleChIP™ Mouse RPL30 Intron 2 Primers #7015. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.**

Entrez-Gene ID #6776, 6777
Swiss-Prot Acc. #P42229, P51692

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μ g/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

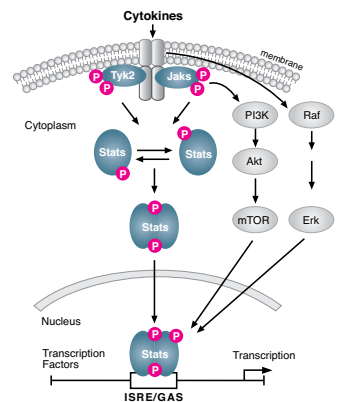
***Species cross-reactivity is determined by western blot.**
****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000
Flow Cytometry	1:400
Chromatin IP	1:50

For application specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.



IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

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Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine
 Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.