#9167 Store at –20°C

Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb

☐ Small 100 µl (10 western blots)

☐ Large 300 µl (30 western blots)

rev. 07/26/12

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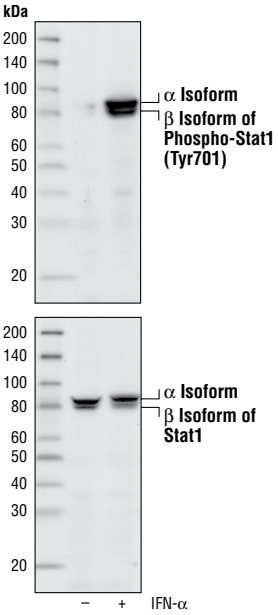
Entrez-Gene ID #6772
Swiss-Prot Acc. #P42224

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IHC-F, IF-IC, F, ChIP Endogenous	H, M	84, 91 kDa	Rabbit IgG**

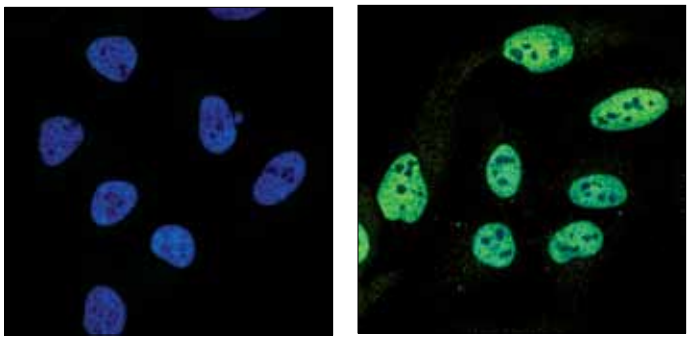
Background: Stat1, while activated in response to a large number of ligands (1), appears to be essential for responsiveness to IFN-α and IFN-γ (2,3). Phosphorylation of Stat1 at Tyr701 induces Stat1 dimerization, nuclear translocation and DNA binding (4). Stat1 has two isoforms, Stat1α (91 kDa) and the splice variant Stat1β (84 kDa). In most cells, both isoforms are activated by IFN-α, but only Stat1α is activated by IFN-γ. Stat1 has been found to be inappropriately activated in many tumors (5). In addition to tyrosine phosphorylation, Stat1 is phosphorylated through a p38 mitogen-activated protein kinase (MAPK)-dependent pathway at Ser727 in response to IFN-α and other cellular stresses (6). Serine phosphorylation may be required for the maximal induction of Stat1-mediated gene activation.

Specificity/Sensitivity: Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb detects endogenous levels of Stat1 only when phosphorylated at tyrosine 701. The antibody detects phosphorylated tyrosine 701 of p91 Stat1 and also the p84 splice variant. It does not cross-react with the corresponding phospho-tyrosines of other Stat proteins.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Tyr701 of human Stat1.



Western blot analysis of extracts from HeLa cells untreated or treated with interferon-α, using Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb (upper) or Stat1 Antibody (#9172) (lower).



Confocal immunofluorescent analysis of HeLa cells, untreated (left) or IFNα-treated #9906 (right), using Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

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.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. 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
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:400†
Unmasking buffer:	Citrate
Unmasking buffer:	EDTA
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114
Immunohistochemistry (Frozen)	1:200†
Fixative:	10% Neutral buffered formalin
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114

†Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.

Immunofluorescence (IF-IC)	1:400
IF Protocol	Methanol Permeabilization required
Flow Cytometry	1:200
Chromatin IP	1:100

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.

Background References:

(1) Heim, M.H. (1999) *J. Recept. Signal. Transduct. Res.* 19, 75–120.

(2) Durbin, J.E. et al. (1996) *Cell* 84, 443–450.

(3) Meraz, M.A. et al. (1996) *Cell* 84, 431–442.

(4) Ihle, J.N. et al. (1994) *Trends Biochem. Sci.* 19, 222–227.

(5) Frank, D.A. (1999) *Mol. Med.* 5, 432–456.

(6) Wen, Z. et al. (1995) *Cell* 82, 241–250.

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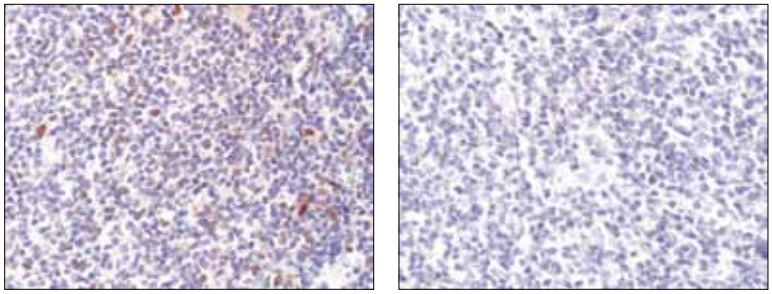
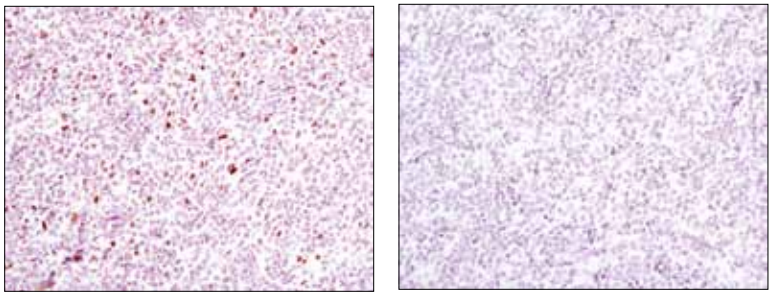
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

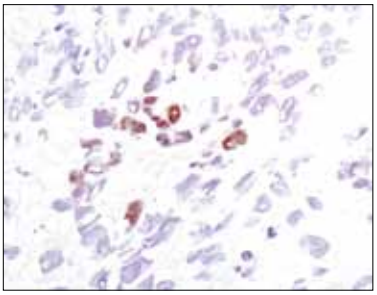
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



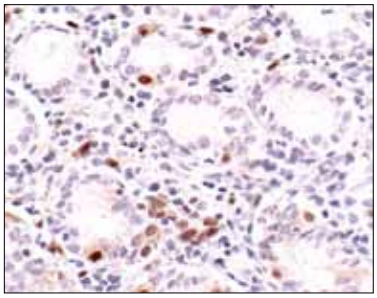
Immunohistochemical analysis of paraffin-embedded Non-Hodgkin's lymphoma control (left) or λ phosphatase treated (right), using Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb.



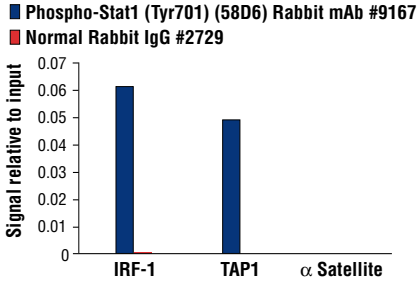
Immunohistochemical analysis of paraffin-embedded Non-Hodgkin's lymphoma using Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb #9167 in the presence of control peptide (left) or Phospho-Stat1 (Tyr701) Blocking Peptide (right).



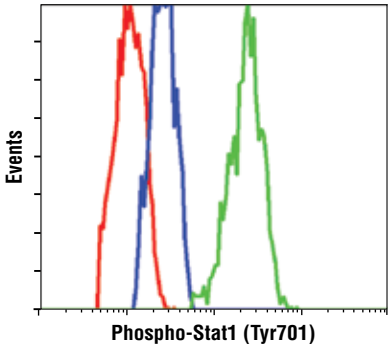
Immunohistochemical analysis of frozen SKOV-3 xenograft using Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded stomach (chronic gastritis), using Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb.



Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10^6 HT-1080 cells treated with IFN-γ (50 ng/ml) for 30 minutes and either 5 µl of Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by Real-Time PCR using human IRF-1 promoter primers, SimpleChIP® Human TAP1 Promoter Primers #5148, and SimpleChIP® Human α Satellite Repeat Primers #4486. 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.



Flow cytometric analysis of Hela cells, untreated (blue) or IFN-α treated (green), using Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb compared to a nonspecific negative control antibody (red).

Immunohistochemical analysis using Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb on SignalSlide® Phospho-Stat1/3/5 IHC Controls #8105 (paraffin-embedded HeLa cell pellets, untreated (left), treated with Human Interferon-α1 (hIFN-α1) #8927 (middle), or treated with Human Epidermal Growth Factor (hEGF) #8916 (right).

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