

Phospho-Stat3 (Tyr705) (D3A7) XP[®] Rabbit mAb

- Small 100 µl (20 western blots)
- Large 300 µl (60 western blots)
- Petite 40 µl (8 western blots)

rev. 01/23/12



Orders ■ 877-616-CELL (2355)
orders@cellsignal.com

Support ■ 877-678-TECH (8324)
info@cellsignal.com

Web ■ www.cellsignal.com

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.

Entrez-Gene ID #6774
Swiss-Prot Acc. #P40763

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IHC-F, IF-IC, F, ChIP Endogenous	H, M, R, Mk, (Hr, Hm, B, Pg)	79, 86 kDa	Rabbit IgG**

Background: Stat3 is a key signaling molecule for many cytokines and growth-factor receptors (1), and is required for murine fetal development (2). Additionally, Stat3 is constitutively activated in a number of human tumors (3,4) and possesses oncogenic potential (5) and anti-apoptotic activities (3). Stat3 is activated by phosphorylation at Tyr705, which induces dimerization, nuclear translocation and DNA binding (6,7). Transcriptional activation seems to be regulated by phosphorylation at Ser727 via the MAPK or mTOR pathway (8,9). Stat3 isoform expression appears to reflect biological function: the relative expression levels of Stat3 α (86 kDa) and Stat3 β (79 kDa) depend on cell type, ligand exposure or maturation stage of the cells (10). It is notable that Stat3 β lacks the serine phosphorylation site within the carboxy-terminal transcriptional activation domain (8).

Specificity/Sensitivity: Phospho-Stat3 (Tyr705) (D3A7) XP[®] Rabbit mAb detects endogenous levels of Stat3 only when phosphorylated at tyrosine 705. This antibody does not cross-react with phospho-EGFR or 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 Tyr705 of mouse Stat3.

Background References:

- Heim, M.H. (1999) *J. Recept. Signal Transduct. Res.* 19, 75–120.
- Takeda, K. et al. (1997) *Proc. Natl. Acad. Sci. USA* 94, 3801–227.
- Catlett-Falcone, R. et al. (1999) *Immunity* 10, 105–115.
- Garcia, R. and Jove, R. (1998) *J. Biomed. Sci.* 5, 79–85.
- Bromberg, J.F. et al. (1999) *Cell* 98, 295–303.
- Darnell Jr., J.E. et al. (1994) *Science* 264, 1415–1421.
- Ihle, J.N. (1995) *Nature* 377, 591–594.
- Wen, Z. et al. (1995) *Cell* 82, 241–250.
- Yokogami, K. et al. (2000) *Curr. Biol.* 10, 47–50.
- Biethahn, S. et al. (1999) *Exp. Hematol.* 27, 885–894.

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.

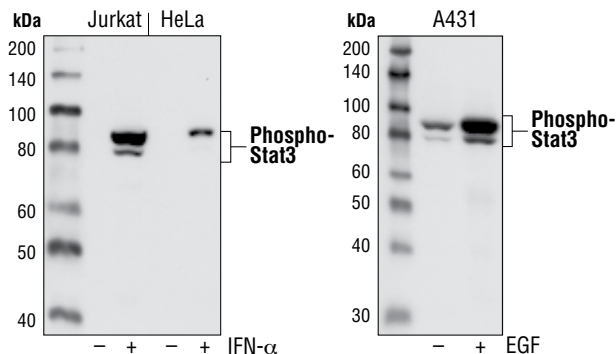
**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

Western blotting	1:2000
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:400†
Unmasking buffer:	EDTA
Antibody diluent:	SignalStain [®] Antibody Diluent #8112
Detection reagent:	SignalStain [®] Boost (HRP, Rabbit) #8114
Immunohistochemistry (Frozen)	1:100†
Fixative	3% Formaldehyde
Detection reagent:	SignalStain [®] Boost (HRP, Rabbit) #8114
†Optimal IHC dilutions determined using SignalStain [®] Boost IHC Detection Reagent.	
Immunofluorescence (IF-IC)	1:100
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.



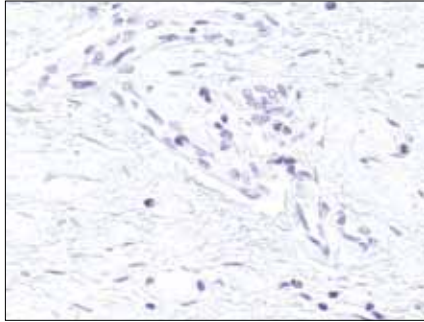
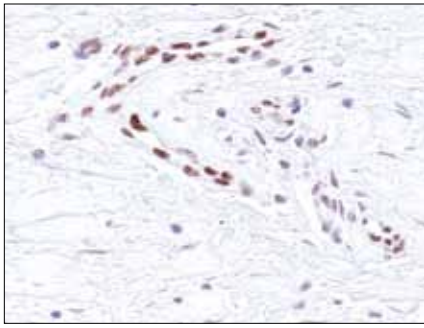
Western blot analysis of extracts from IFN- α treated Jurkat cells and HeLa cells (left), as well as EGF treated A431 cells (right), using Phospho-Stat3 (Tyr705) (D3A7) XP[®] Rabbit mAb. Note that the basal phospho-Stat3 in A431 is detected by the antibody.

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.

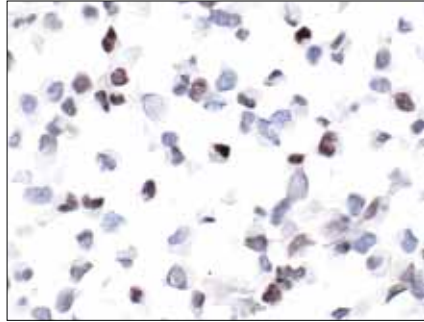
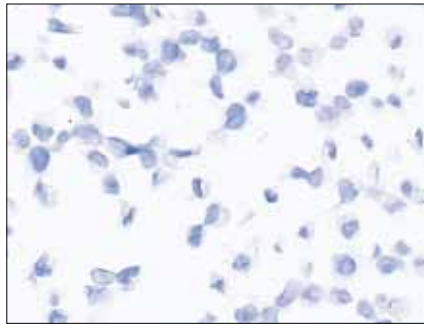
Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide

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

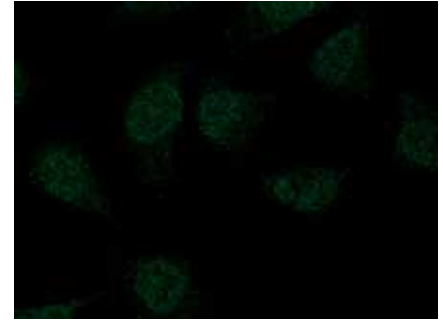
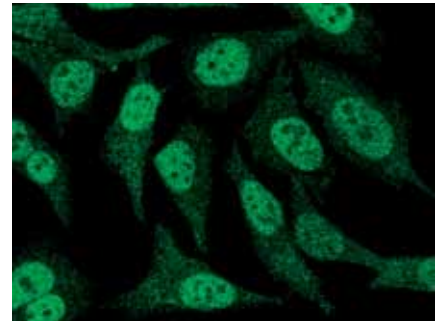
Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



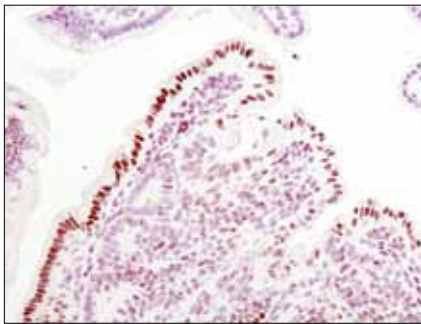
Immunohistochemical analysis of paraffin embedded human breast carcinoma, specifically endothelial cells, untreated (upper) or λ -phosphatase treated (lower), using Phospho-Stat3 (Tyr705) (D3A7) XP[®] Rabbit mAb.



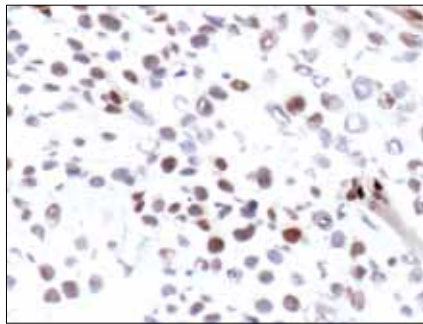
Immunohistochemical analysis using Phospho-Stat3 (Tyr705) (D3A7) XP[®] Rabbit mAb on SignalSlide™ Phospho-Stat 1/3/5 IHC Controls #8105 (paraffin-embedded 786-0 cells, untreated (upper) or IFN- α treated (lower)).



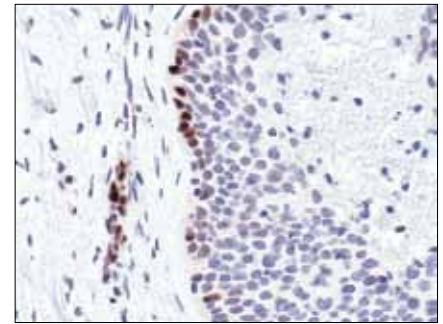
Confocal immunofluorescent analysis of HeLa cells, IFN- α treated (upper) or untreated (lower), labeled with Phospho-Stat3 (Tyr705) (D3A7) XP[®] Rabbit mAb (green).



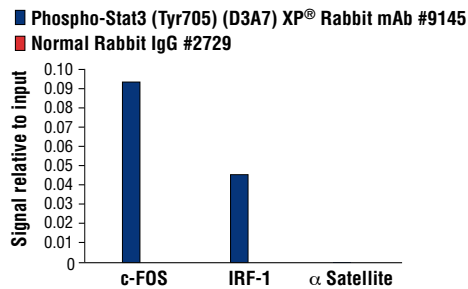
Immunohistochemical analysis of paraffin-embedded Apc (min/+) mouse intestine, using Phospho-Stat3 (Tyr705) (D3A7) XP[®] Rabbit mAb.



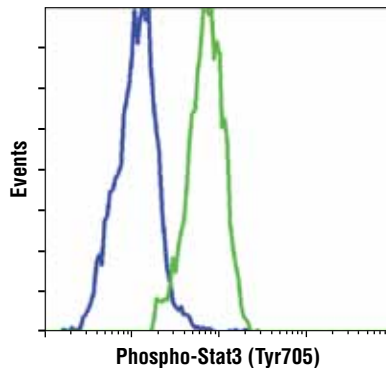
Immunohistochemical analysis of frozen H1650 xenograft using Phospho-Stat3 (Tyr705) (D3A7) XP[®] Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human lung carcinoma, showing nuclear localization, using Phospho-Stat3 (Tyr705) (D3A7) XP[®] Rabbit mAb.



Chromatin immunoprecipitations were performed with cross-linked chromatin from 4×10^6 Hep G2 cells starved overnight and treated with IL-6 (100 ng/ml) for 30 minutes, and either 10 μ l of Phospho-Stat3 (Tyr705) (D3A7) XP[®] Rabbit mAb 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 human IRF-1 promoter primers, SimpleChIP[®] Human c-FOS Promoter Primers #4663, 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-Stat3 (Tyr705) (D3A7) XP[®] Rabbit mAb.