

#9411

Store at -20°C

# Phospho-Tyrosine Mouse mAb (P-Tyr-100)

✓ 300 µg  
(40 western blots)



**Orders** ■ 877-616-CELL (2355)  
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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*	Isotype
W, IP, IHC-P, IF-F, IF-IC, IF-P, F, E-P Endogenous	All	Mouse IgG1**

**Background:** Tyrosine phosphorylation plays a key role in cellular signaling (1). In cancer, unregulated tyrosine kinase activity can drive malignancy and tumor formation by generating inappropriate proliferation and survival signals (2). Antibodies specific for phospho-tyrosine (3,4) have been invaluable reagents in these studies. The phospho-tyrosine monoclonal antibodies developed by CST provide exceptionally sensitive new tools of increased utility for studying tyrosine phosphorylation and monitoring tyrosine kinase activity in high throughput drug discovery.

**Specificity/Sensitivity:** Phospho-Tyrosine Mouse mAb (P-Tyr-100) is a high affinity antibody. ELISAs against a wide variety of phosphopeptides indicate that P-Tyr-100 binds phospho-Tyr in a manner largely independent of the surrounding amino acid sequence. 2D gel Western blot analysis of pervanadate-treated cell extracts also shows that P-Tyr-100 interacts with a broad range of tyrosine-phosphorylated proteins. P-Tyr-100 does not cross-react with peptides containing phospho-Ser or phospho-Thr. (U.S. Patent No.'s.: 6,441,140; 6,982,318; 7,259,022; 7,344,714; U.S.S.N. 11,484,485; and all foreign equivalents.)

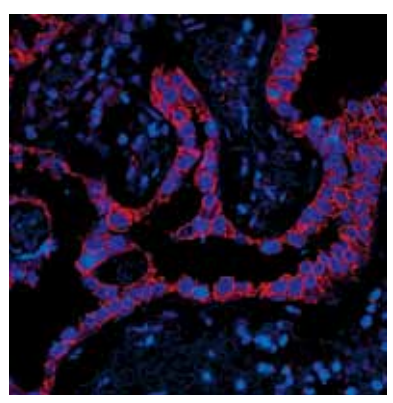
**Source/Purification:** Monoclonal antibody is produced by immunizing animals with phospho-tyrosine containing peptides.

**Background References:**

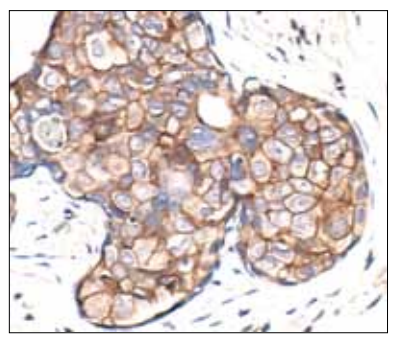
- Schlessinger, J. (2000) *Cell* 103, 211-225.
- Blume-Jensen, P. and Hunter, T. (2001) *Nature* 411, 355-365.
- Ward, S.G. et al. (1992) *J. Biol. Chem.* 267, 23862-23869.
- Glenney, J.R. et al. (1988) *J. Immunol. Methods.* 109, 277-285.

**License/Use Restrictions:** Use of CST Motif Antibodies within certain methods (e.g., U.S. Patent No.'s 7,198,896 & 7,300,753) may require a license from CST. For information regarding academic licensing terms please have your technology transfer office contact CST Legal Department at CST\_ip@cellsignal.com. For information regarding commercial licensing terms please contact CST Pharma Services Department at ptmscan@cellsignal.com.

**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.**



Confocal immunofluorescent image of paraffin-embedded human lung adenocarcinoma labeled with Phospho-Tyrosine Mouse mAb (P-Tyr-100) (red). Blue pseudocolor = DRAQ5® (fluorescent DNA dye).



Immunohistochemical analysis of paraffin-embedded human breast carcinoma, showing staining of proteins with phosphorylated tyrosine residues, using Phospho-Tyrosine Mouse mAb(P-Tyr-100).

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

\*Species cross-reactivity is determined by western blot.

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

**Recommended Antibody Dilutions:**

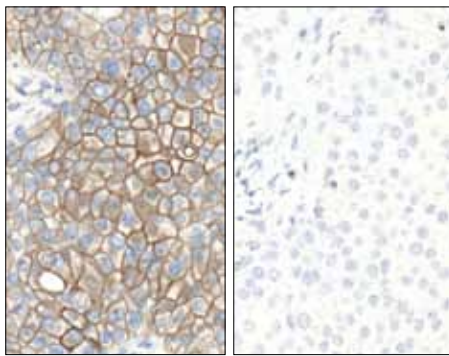
Western Blotting	1:2000
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:4800†
Unmasking buffer:	EDTA
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Mouse) #8125
† Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.	
Immunofluorescence (IF-F)	1:1600
Immunofluorescence (IF-IC)	1:1600
Immunofluorescence (IF-P)	1:1600
ELISA-Peptide	1:4000
Flow Cytometry	1:3200

For application specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

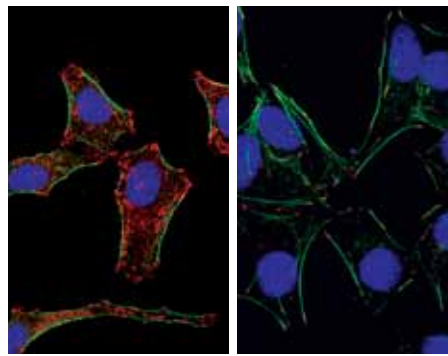
Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

DRAQ5® is a registered trademark of Biostatus Limited.

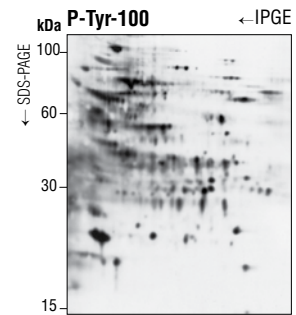
**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish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



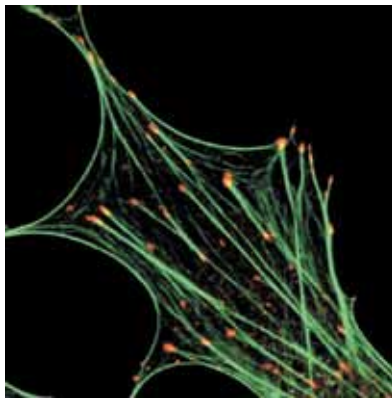
Immunohistochemical analysis of paraffin-embedded NCI-H1650 xenograft untreated (left) or  $\lambda$ -phosphatase-treated (right), using Phospho-Tyrosine Mouse mAb (P-Tyr-100).



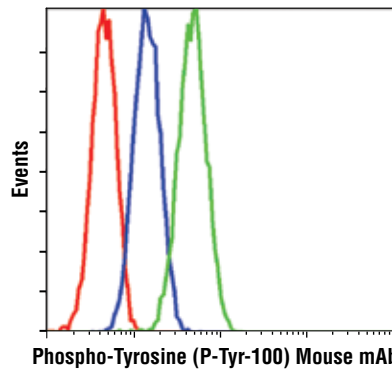
Confocal immunofluorescent images of HeLa cells 20% serum-treated (left) or untreated (right) and labeled with Phospho-Tyrosine Mouse mAb (P-Tyr-100) (red). Actin filaments have been labeled with fluorescein phalloidin. Blue pseudocolor = DRAQ5™ (fluorescent DNA dye).



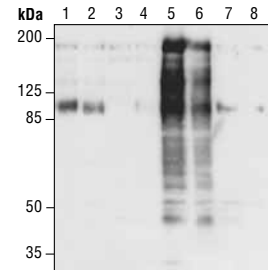
P-Tyr-100 Phospho-Tyrosine Mouse Monoclonal Antibody: Western blot analysis of extracts from Jurkat cells treated with 1 mM pervanadate for 30 minutes prior to lysis. Proteins were separated by 2-D electrophoresis prior to blotting.



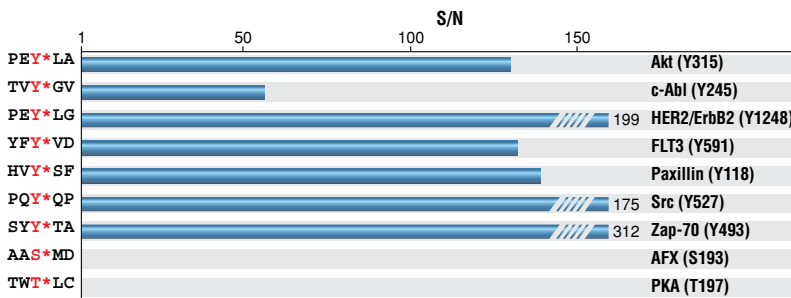
Immunofluorescent analysis of serum-starved Swiss NIH/3T3 cells, stimulated with lysophosphatidic acid (LPA) (10  $\mu$ M for 10 minutes), fixed with PFA and stained with phalloidin for F-actin (green) and Phospho-Tyrosine Mouse mAb (P-Tyr-100) (red). LPA causes heavy tyrosine phosphorylation of proteins in focal adhesions, present at the tips of actin stress fibers. (Provided by Dr. Harry Mellor, University of Bristol.)



Flow cytometric analysis of K562 cells, untreated (green) or Gleevec®- treated (blue), using Phospho-Tyrosine Mouse mAb (P-Tyr-100) compared with a nonspecific negative control antibody (red).



Tyrosine phosphorylation in rat hippocampal dentate gyrus ischemia. Control (lanes 1 and 2); 15 minute transient cerebral ischemia (lanes 3 and 4); reperfusion for 4 hours (lanes 5 and 6); and 24 hours (lanes 7 and 8). Western blot analysis using Phospho-Tyrosine Mouse mAb (P-Tyr-100). (Provided by Dr. Bingren Hu, Queen's Medical Center, Hawaii.)



Phospho-Tyrosine Mouse mAb (P-Tyr-100) ELISA Assay: Signal-to-noise ratio of phospho- versus nonphospho-peptides. (Y\* denotes phosphorylated tyrosine.)