#12571 Store at -20°C

Phospho-p53 (Ser15) (D4S1H) XP® Rabbit mAb (Rodent Specific)

- ☐ Small 100 µl (10 western blots)
- ☐ Large 300 µl (30 western blots)
- ☐ Petite 40 µl (4 western blots)

rev. 01/15/15

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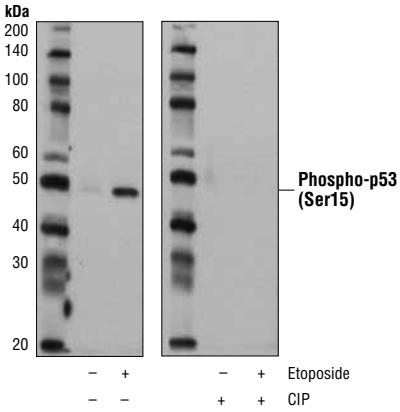
Entrez-Gene ID #22059
UniProt ID #P02340

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IF-IC, F Endogenous	M, R	53 kDa	Rabbit IgG**

Background: The p53 tumor suppressor protein plays a major role in cellular response to DNA damage and other genomic aberrations. Activation of p53 can lead to either cell cycle arrest and DNA repair or apoptosis (1). p53 is phosphorylated at multiple sites *in vivo* and by several different protein kinases *in vitro* (2,3). DNA damage induces phosphorylation of p53 at Ser15 and Ser20 and leads to a reduced interaction between p53 and its negative regulator, the oncoprotein MDM2 (4). MDM2 inhibits p53 accumulation by targeting it for ubiquitination and proteasomal degradation (5,6). p53 can be phosphorylated by ATM, ATR, and DNA-PK at Ser15 and Ser37. Phosphorylation impairs the ability of MDM2 to bind p53, promoting both the accumulation and activation of p53 in response to DNA damage (4,7). Chk2 and Chk1 can phosphorylate p53 at Ser20, enhancing its tetramerization, stability, and activity (8,9). p53 is phosphorylated at Ser392 *in vivo* (10,11) and by CAK *in vitro* (11). Phosphorylation of p53 at Ser392 is increased in human tumors (12) and has been reported to influence the growth suppressor function, DNA binding, and transcriptional activation of p53 (10,13,14). p53 is phosphorylated at Ser6 and Ser9 by CK1δ and CK1ε both *in vitro* and *in vivo* (13,15). Phosphorylation of p53 at Ser46 regulates the ability of p53 to induce apoptosis (16). Acetylation of p53 is mediated by p300 and CBP acetyltransferases. Inhibition of deacetylation suppressing MDM2 from recruiting HDAC1 complex by p19 (ARF) stabilizes p53. Acetylation appears to play a positive role in the accumulation of p53 protein in stress response (17). Following DNA damage, human p53 becomes acetylated at Lys382 (Lys379 in mouse) *in vivo* to enhance p53-DNA binding (18). Deacetylation of p53 occurs through interaction with the SIRT1 protein, a deacetylase that may be involved in cellular aging and the DNA damage response (19).

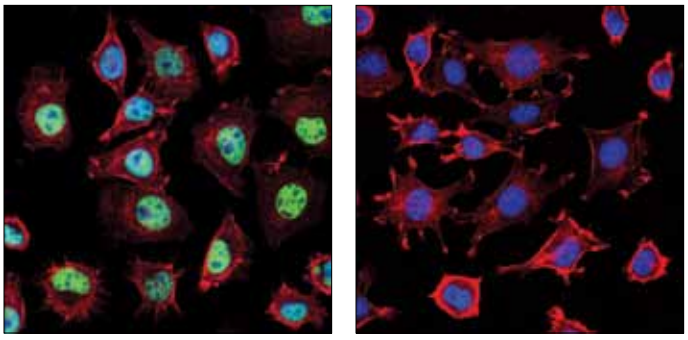
Specificity/Sensitivity: Phospho-p53 (Ser15) (D4S1H) XP® Rabbit mAb (Rodent Specific) recognizes endogenous levels of p53 protein only when phosphorylated at Ser15.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser15 of mouse p53 protein.



Western blot analysis of extracts from L-929 cells, untreated or treated with Etoposide #2200 (30 µM, 2 hours), using Phospho-p53 (Ser15) (D4S1H) XP® Rabbit mAb (Rodent Specific). To characterize the phospo-specificity of the antibody, the blot was mock treated (-) or treated (+) with calf intestinal phosphatase (CIP).

Etoposide-treated **Untreated**



Confocal immunofluorescent analysis of L-929 cells, untreated (right) or treated with Etoposide #2200 (left), using Phospho-p53 (Ser15) (D4S1H) XP® Rabbit mAb (Rodent Specific) (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

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	1:50
Immunohistochemistry (Paraffin)	1:3200†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114

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

Immunofluorescence (IF-IC)	1:3200
Flow Cytometry	1:3200

For product 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 nonfat dry milk, 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.

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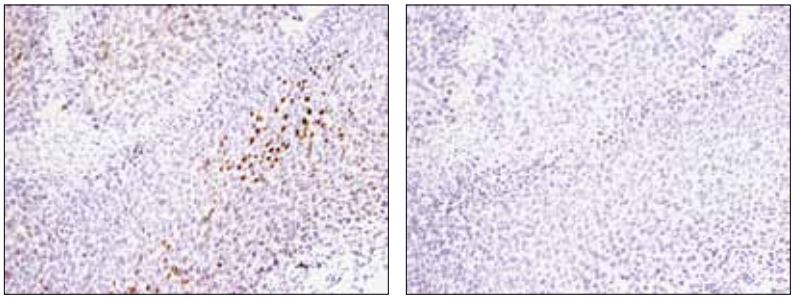
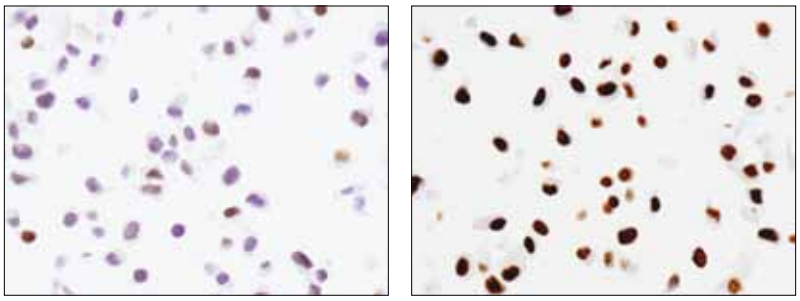
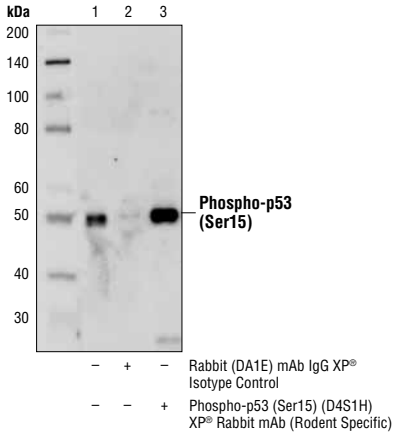


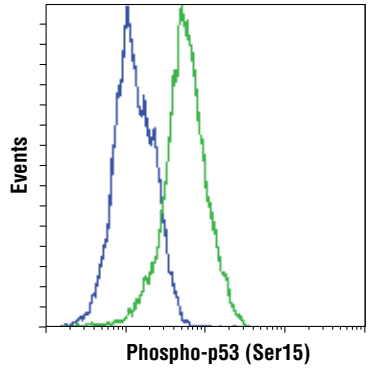
Immunohistochemical analysis of paraffin-embedded LL/2 syngeneic tumor, untreated (left) or λ phosphatase-treated (right), using Phospho-p53 (Ser15) (D4S1H) XP® Rabbit mAb (Rodent Specific).



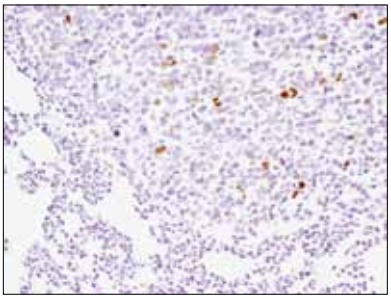
Immunohistochemical analysis of paraffin-embedded L-929 cell pellets, untreated (left) or treated with Etoposide #2200 (right), using Phospho-p53 (Ser15) (D4S1H) XP® Rabbit mAb (Rodent Specific).



Immunoprecipitation of phospho-p53 (Ser15) from L-929 cell extracts treated with Etoposide #2200 (30 µM, 2 hr) using Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (lane 2) or Phospho-p53 (Ser15) (D4S1H) XP® Rabbit mAb (Rodent Specific) (lane 3). Lane 1 is 10% input. Western blot analysis was performed using Phospho-p53 (Ser15) (D4S1H) XP® Rabbit mAb (Rodent Specific). A light-chain specific antibody was used as a secondary antibody.



Flow cytometric analysis of L-929 cells, untreated (blue) or treated with Etoposide #2200 (30 µM, 2 hr; green), using Phospho-p53 (Ser15) (D4S1H) XP® Rabbit mAb (Rodent Specific). Anti-rabbit IgG (H+L), F(ab')2 fragment (Alexa Fluor® 488 conjugate) #4412 was used as a secondary antibody.



Immunohistochemical analysis of paraffin-embedded 4T1 metastatic tumors in mouse lung using Phospho-p53 (Ser15) (D4S1H) XP® Rabbit mAb (Rodent Specific). Note lack of staining in adjacent normal lung.

Background References:

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