

#2524 Store at -20°C

# p53 (1C12) Mouse mAb



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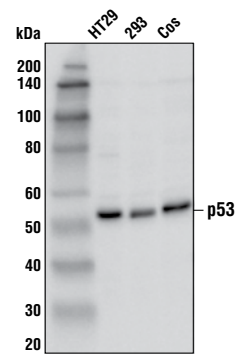
Entrez-Gene ID #7157  
UniProt ID #P04637

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IF-IC, F, ChIP Endogenous	H, M, R, Mk, Hm	53 kDa	Mouse IgG1 κ**

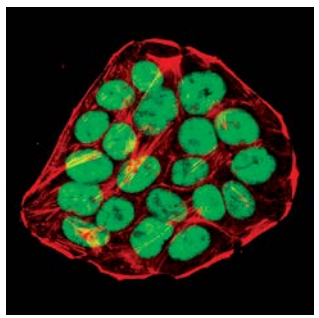
**Background:** The p53 tumor suppressor protein plays a major role in cellular response to DNA damage and other genomic aberrations. Activation of p53 can lead to either cell cycle arrest and DNA repair or apoptosis (1). p53 is phosphorylated at multiple sites *in vivo* and by several different protein 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:** p53 (1C12) Mouse mAb detects endogenous levels of total p53 protein.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser20 of human p53.



Western blot analysis of cellular extracts using p53 (1C12) Mouse mAb.



Confocal immunofluorescent analysis of HT-29 cells using p53 (1C12) Mouse mAb (green). Actin filaments have been labeled with DyLight™ 554 Phalloidin #13054 (red).

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**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.**

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

**Recommended Antibody Dilutions:**

Western blotting	1:1000
Immunoprecipitation	1:500
Immunofluorescence (IF-IC)	1:4000
Flow Cytometry	1:3200
Chromatin IP	1:200

Optimal ChIP conditions: 2.5 µl of antibody & 10 µg of chromatin (4 x 10<sup>6</sup> cells) per IP. Antibody validated using SimpleChIP® Enzymatic ChIP Kits.

For product specific protocols and a complete listing of recommended companion products please see the product web page at [www.cellsignal.com](http://www.cellsignal.com).

**Background References:**

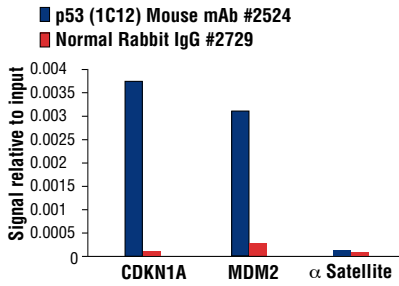
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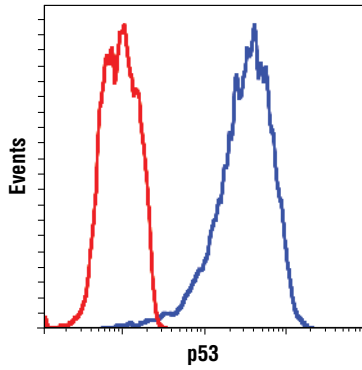
**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide

**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.



Chromatin immunoprecipitations were performed with cross-linked chromatin from HCT116 cells treated with UV (100 J/m<sup>2</sup> followed by a 3 hour recovery) and either p53 (1C12) Mouse mAb or Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human CDKN1A Promoter Primers #6449, human MDM2 intron 2 primers, and SimpleChIP® Human  $\alpha$ -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 HT-29 cells using p53 (1C12) Mouse mAb (blue) compared to concentration matched Mouse (G3A1) mAb IgG1 Isotype Control #5415 (red). Anti-mouse IgG (H+L), F(ab')<sub>2</sub> Fragment (Alexa Fluor® 488 Conjugate) #4408 was used as a secondary Ab.