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p53 Antibody



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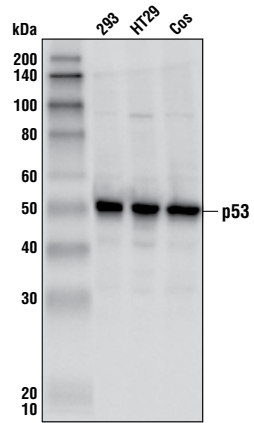
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Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, ChIP Endogenous	H, Mk	53 kDa	Rabbit**

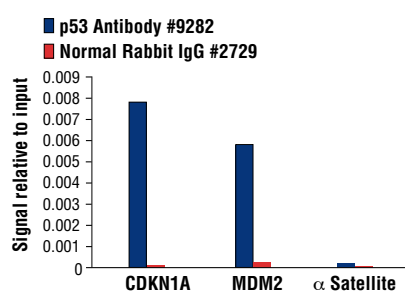
Background: The p53 tumor suppressor protein plays a major role in cellular response to DNA damage and other genomic aberrations. Activation of p53 can lead to either cell cycle arrest and DNA repair or apoptosis (1). p53 is phosphorylated at multiple sites *in vivo* and by several different protein 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 CK1 δ and CK1 ϵ 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 Antibody recognizes endogenous levels of total p53 protein. This antibody binding has been mapped to the amino terminus and DNA binding domain of human p53 protein. This antibody does not cross-react with other p53-related proteins.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a full-length human p53 fusion protein. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of cell extracts using p53 Antibody



Chromatin immunoprecipitations were performed with cross-linked chromatin from HCT116 cells treated with UV (100 J/m² followed by a 3 hour recovery) and either p53 Antibody 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 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.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% nonfat dry milk, 1X TBS, 0.1% Tween[®]20 at 4°C with gentle shaking, overnight.

Entrez-Gene ID #7157
UniProt ID #P04637

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 $\mu\text{g/ml}$ BSA and 50% glycerol. 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
Chromatin IP	1:100

Optimal ChIP conditions: 5 μl of antibody & 10 μg of chromatin (4 x 10⁶ cells) per IP. Antibody validated using SimpleChIP[®] Enzymatic ChIP Kits.

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:

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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.