

#2525
 Store at -20°C

Acetyl-p53 (Lys382) Antibody

- Small 100 µl
(10 Western mini-blot)
- Large 300 µl
(30 Western mini-blot)

Orders ■ 877-616-CELL (2355)
 orders@cellsignal.com
Support ■ 877-678-TECH (8324)
 info@cellsignal.com
Web ■ www.cellsignal.com

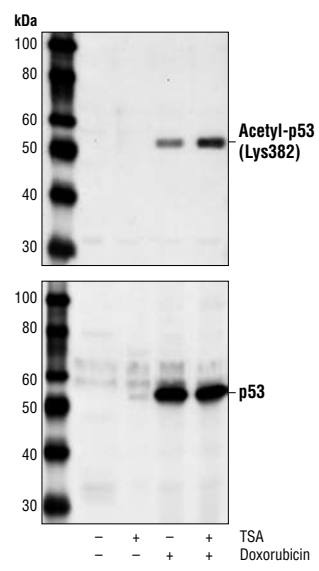
rev. 03/07/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W	H	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 reduced interaction of p53 with its negative regulator, oncoprotein MDM2 (4). MDM2 inhibits the accumulation of p53 by targeting it for ubiquitination and proteasomal degradation (6,7). p53 can apparently be phosphorylated by ATM, ATR and DNA-PK at Ser15 and Ser37; the phosphorylations impair the ability of MDM2 to bind p53, promoting both the accumulation and activation of p53 in response to DNA damage (4,5). Chk2 and Chk1 can phosphorylate p53 at Ser20, enhancing its tetramerization, stability and activity (8,9). p53 is phosphorylated at Ser392 *in vivo* (11,12) and by CAK *in vitro* (12). Phosphorylation of p53 at Ser392 is altered in human tumors (14) and has been reported to influence the growth suppressor function, DNA binding and transcriptional activation of p53 (10,11,13). p53 is phosphorylated at Ser6 and Ser9 by ck1δ and ck1ε both *in vitro* and *in vivo* (10,15). Phosphorylation of p53 at Ser46 is important in regulating 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).

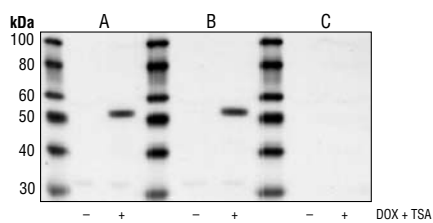
The histone acetyltransferases p300 and PCAF can acetylate p53 *in vitro* at Lys382 and Lys320, respectively (17). Lys382 becomes acetylated *in vivo* following DNA damage to allow enhanced p53-DNA binding (18).



Western blot analysis of extracts from HeLa cells, untreated, trichostatin A-treated #9950 (400 nM for 24 hours), doxorubicin-treated (0.5 µM for 24 hours), or both, using Acetyl-p53 (Lys382) Antibody (top) or p53 Antibody #2524 (bottom).

Specificity/Sensitivity: Acetyl-p53 (Lys382) Antibody detects endogenous levels of p53 only when acetylated at lysine 382. This antibody does not cross-react with other acetylated proteins.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic acetylated peptide (KLH-coupled) corresponding to residues surrounding Lys382 of human p53. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from HeLa cells, untreated or treated with both trichostatin A #9950 (400 nM for 24 hours), and doxorubicin (0.5 µM for 24 hours) using Acetyl-p53 (Lys382) Antibody alone (A), antibody preincubated with a non-acetylated Lys382 peptide (B), or antibody preincubated with an acetylated Lys382 peptide (C).

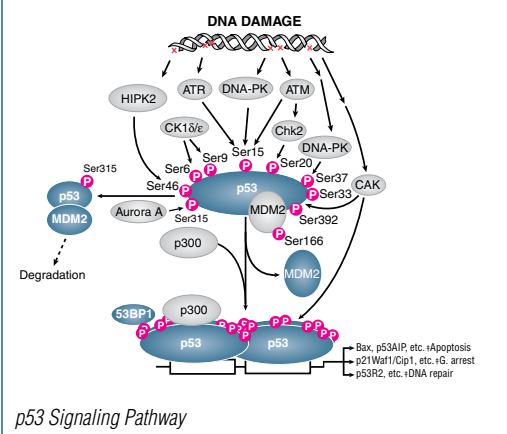
Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by Western blot.

Recommended Antibody Dilutions:
Western Blotting 1:1000

- Companion Products:**
- p53 Antibody #9282
 - p53 (1C12) Mouse mAb #2524
 - SignalSilence® p53 siRNA Kit (Human Specific) #6230
 - Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
 - Anti-rabbit IgG, HRP-linked Antibody #7074
 - Prestained Protein Marker, Broad Range (Premixed Format) #7720
 - Biotinylated Protein Ladder Detection Pack #7727
 - 20X LumiGLO® Reagent and 20X Peroxide #7003

IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.



p53 Signaling Pathway

Background References:

- (1) Levine, A.J. (1997) *Cell* 88, 323–331.
- (2) Meek, D.W. (1994) *Semin. Cancer Biol.* 5, 203–210.
- (3) Milczarek, G.J. et al. (1997) *Life Sci.* 60, 1–11.
- (4) Shieh, S.Y. et al. (1997) *Cell* 91, 325–334.
- (5) Tibbetts, R.S. et al. (1999) *Genes Dev.* 13, 152–157.
- (6) Chehab, N.H. et al. (1999) *Proc. Natl. Acad. Sci. USA* 96, 13777–13782.
- (7) Honda, R. et al. (1997) *FEBS Lett.* 420, 25–27.
- (8) Shieh, S.Y. et al. (1999) *EMBO J.* 18, 1815–1823.
- (9) Hirao, A. et al. (2000) *Science* 287, 1824–1827.
- (10) Kohn, K.W. (1999) *Mol. Biol. Cell* 10, 2703–2734.
- (11) Hao, M. et al. (1996) *J. Biol. Chem.* 271, 29380–29385.
- (12) Lu, H. et al. (1997) *Mol. Cell. Biol.* 17, 5923–5934.
- (13) Lohrum, M. and Scheidtmann, K.H. (1996) *Oncogene* 13, 2527–2539.
- (14) Ulrich, S.J. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90, 5954–5958.
- (15) Knippschild, U. et al. (1997) *Oncogene* 15, 1727–1736.
- (16) Oda, K. et al. (2000) *Cell* 102, 849–862.
- (17) Ito, A. et al. (2001) *EMBO* 20, 1331–1340.
- (18) Gu, W. and Roeder, R.G. (1997) *Cell* 90, 595–606.
- (19) Sakaguchi, K. et al. (1998) *Genes Dev.* 12, 2831–2841.

Western Immunoblotting Protocol (Primary Ab Incubation In BSA)

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.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.