

Phospho-p53 Antibody Sampler Kit



✓ 1 Kit
(9 x 40 µl)

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info@cellsignal.com
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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.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-p53 (Ser6) Antibody	9285	40 µl	53 kDa	Rabbit IgG
Phospho-p53 (Ser9) Antibody	9288	40 µl	53 kDa	Rabbit IgG
Phospho-p53 (Ser15) Antibody	9284	40 µl	53 kDa	Rabbit IgG
Phospho-p53 (Thr81) Antibody	2676	40 µl	53 kDa	Rabbit IgG
Phospho-p53 (Ser20) Antibody	9287	40 µl	53 kDa	Rabbit IgG
Phospho-p53 (Ser37) Antibody	9289	40 µl	53 kDa	Rabbit IgG
Phospho-p53 (Ser46) Antibody	2521	40 µl	53 kDa	Rabbit IgG
Phospho-p53 (Ser392) Antibody	9281	40 µl	53 kDa	Rabbit IgG
p53 (7F5) Rabbit mAb	2527	40 µl	53 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The Phospho-p53 Antibody Sampler Kit provides a fast and economical means of evaluating multiple phosphorylation sites of p53 protein. The kit contains enough primary and secondary antibodies to perform four Western blot experiments.

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 (Ser6), (Ser9), (Ser15), (Ser20), (Ser37), (Thr81), and (Ser392) Antibodies detect p53 only when phosphorylated at the indicated sites and do not cross-react with p53 phosphorylated at other sites.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser6, Ser9, Ser15, Ser20, Ser37, Thr81, and Ser392 of human p53. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibody is produced by immunizing animals with a MBP-p53 fusion protein.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibodies.

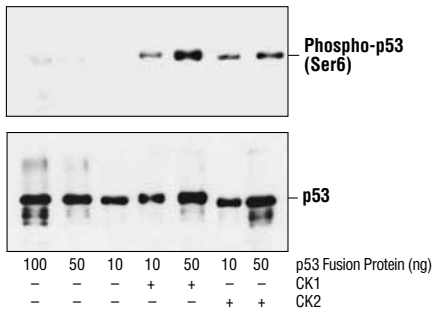
Recommended Antibody Dilutions:
Western blotting 1:1000

Please visit www.cellsignal.com for a complete listing of recommended companion products.

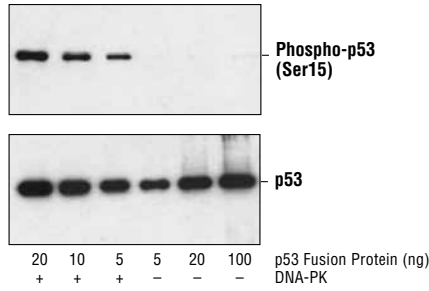
Background References:

- (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) Ullrich, 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 J.* 20, 1331–1340.
- (18) Sakaguchi, K. et al. (1998) *Genes Dev.* 12, 2831–2841.
- (19) Solomon, J.M. et al. (2006) *Mol. Cell. Biol.* 26, 28–38.

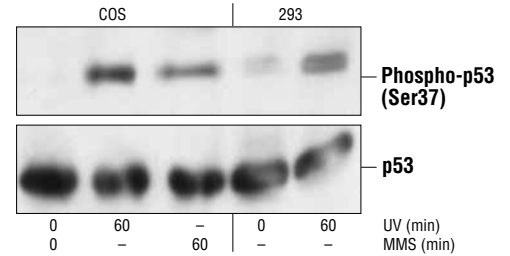
Selected rabbit monoclonal antibodies are produced under license (granting certain rights including those under U. S. Patent No. 5,675,063 and/or U.S.S.N. 11/476,277) from Epitomics, Inc. U.S.S.N. 11/476,277) from Epitomics, Inc.



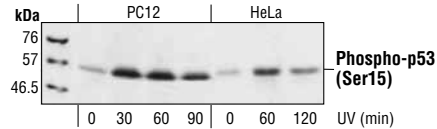
Western blot analysis of p53 fusion protein with and without CKI or CKII phosphorylation, using **Phospho-p53 (Ser6) Antibody #9285** or p53 Antibody #9282.



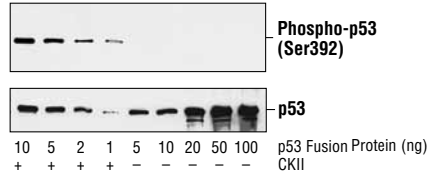
Specificity and sensitivity of **Phospho-p53 (Ser15) Antibody #9284**. Western blot analysis of p53 fusion protein with and without DNA-PK phosphorylation, using Phospho-p53 (Ser15) Antibody (upper) and p53 Antibody #9282 (lower).



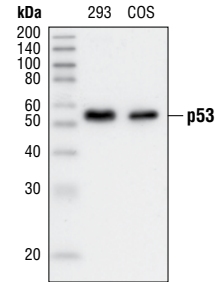
Western blot analysis of extracts from COS cells treated with UV or MMS and 293 cells treated with UV, using **Phospho-p53 (Ser37) #9289** (upper) and p53 Antibody #9282 (lower).



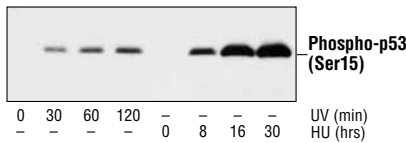
Western blot analysis of extracts from UV treated PC12 cells or HeLa cells, using **Phospho-p53 (Ser15) Antibody #9284**.



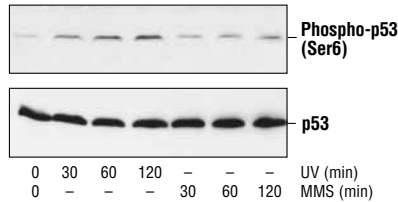
Western blot analysis of p53 fusion protein with and without CKII phosphorylation, using **Phospho-p53 (Ser392) Antibody #9281** or p53 Antibody #9282. Phospho-p53 Antibody reacts specifically with as little as 1 ng of phosphorylated p53 protein.



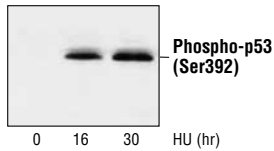
Western blot analysis of extracts from 293 and COS cells, using **p53 (7F5) Rabbit mAb #2527**.



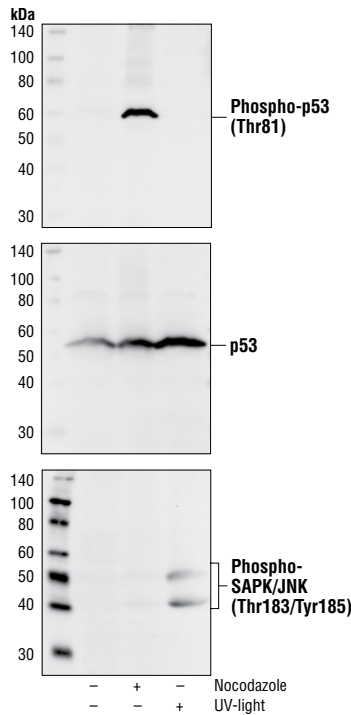
Western blot analysis of extracts from UV or hydroxyurea (20 mM) treated Mv1Lu cells, using **Phospho-p53 (Ser15) Antibody #9284**.



Western blot analysis of extracts from COS cells treated with UV or MMS, using **Phospho-p53 (Ser6) Antibody #9285** or p53 Antibody #9282.



Western blot analysis of extracts from hydroxyurea (20 mM) treated Mv1Lu cells, using **Phospho-p53 (Ser392) Antibody #9281**.



Western blot analysis of extracts from HT29 cells, untreated, nocodazole-treated (50 ng/ml, 24h) or UV-treated (50mJ/cm², 1hr), using **Phospho-p53 (Thr81) Antibody #2676** (upper), p53 (1C12) Mouse mAb #2524 (middle), or Phospho-SAPK/JNK(T183/Y185) (98F2) Rabbit mAb #4671 (lower).

Western Immunoblotting Protocol (Primary Antibody 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.

Western Immunoblotting Protocol (Primary Antibody Incubation in Milk)

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.

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)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope®-HRP Western Blot Detection System #7072:** Includes biotinylated protein ladder, secondary anti-mouse (#7076) 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 marker (#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.