

#9947 Store at -20°C

DNA Damage Antibody Sampler Kit

1 Kit
 (7 x 40 µl)

Orders ■ 877-616-CELL (2355)
 orders@cellsignal.com
Support ■ 877-678-TECH (8324)
 info@cellsignal.com
Web ■ www.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-ATM (Ser1981) (D6H9) Rabbit mAb	5883	40 µl	350 kDa	Rabbit IgG
Phospho-ATR (Ser428) Antibody	2853	40 µl	300 kDa	Rabbit IgG
Phospho-BRCA1 (Ser1524) Antibody	9009	40 µl	220 kDa	Rabbit IgG
Phospho-Chk1 (Ser296) Antibody	2349	40 µl	56 kDa	Rabbit IgG
Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb	9718	40 µl	15 kDa	Rabbit IgG
Phospho-Chk2 (Thr68) Antibody	2661	40 µl	62 kDa	Rabbit IgG
Phospho-p53 (Ser15) (16G8) Mouse mAb	9286	40 µl	53 kDa	Mouse IgG1
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse

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

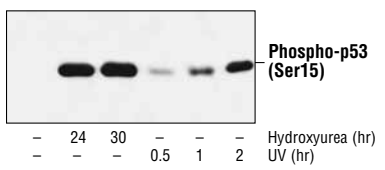
Description: This kit provides an economical means to analyze major signaling checkpoints in response to DNA damage. The kit contains primary and secondary antibodies to perform four Western blots with each antibody.

Background: Ataxia telangiectasia mutated kinase (ATM) and ataxia telangiectasia and Rad3-related kinase (ATR) are PI3 Kinase-related kinase (PIKK) family members that phosphorylate multiple substrates on serine or threonine residues that are followed by a glutamine in response to DNA damage or replication blocks (1-3). p53 is phosphorylated by ATM, ATR and DNA-PK at Ser15. This phosphorylation impairs the ability of MDM2 to bind p53, promoting both the accumulation and activation of p53 in response to DNA damage (4,5). Chk1 and Chk2, downstream protein kinases of ATM/ATR, plays an important role in DNA damage checkpoint control, embryonic development and tumor suppression (6). Chk1 is phosphorylated at Ser280 and Ser296 following DNA damage. The amino-terminal domain of Chk2 contains a series of seven serine or threonine residues, including Thr68, each followed by glutamine (SQ or TQ motif). After DNA damage by ionizing radiation (IR), UV irradiation or hydroxyurea treatment, Thr68 and other sites in this region become phosphorylated by ATM/ATR (7-9). The breast cancer susceptibility proteins BRCA1 and BRCA2 are frequently mutated in cases of hereditary breast and ovarian cancers and have roles in multiple processes related to DNA damage, repair, cell cycle progression, transcription, ubiquitination and apoptosis. Numerous DNA-damage induced phosphorylation sites on BRCA1 have been identified, including serine 1524, and kinases activated in a cell cycle-dependent manner, including Aurora A and CDK2, can also phosphorylate BRCA1. IR, DNA and radiometric-induced DNA damage also results in rapid phosphorylation

of the histone H2A family member H2A.X at Ser139 by ATM (10,11). Within minutes following DNA damage, Ser139-phosphorylated H2A.X localizes to sites of DNA damage at subnuclear foci (12).

Specificity/Sensitivity: All antibodies in the DNA Damage Antibody Sampler Kit recognize their targets proteins only when modified at the indicated site.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser428 of human ATR; Ser1524 of human BRCA1; Ser296 of human Chk1; or Thr68 of human Chk2. Antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding, Ser139 of Histone H2A.X; Ser1981 of human ATM; or Ser15 of human p53.



Western blot analysis of extracts from Mv1Lu cells, untreated, hydroxyurea-treated (20 mM) or UV-treated, using **Phospho-p53 (Ser15) (16G8) Mouse mAb #9286**.

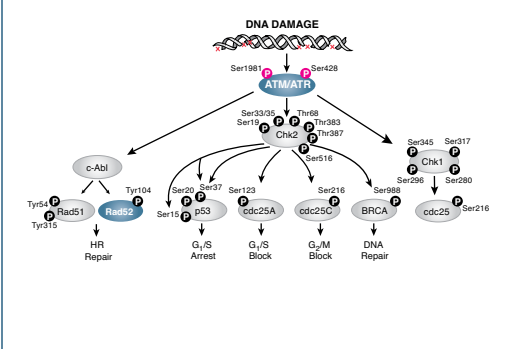
Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibodies.

Recommended Antibody Dilutions:
Western blotting 1:1000

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

Background References:

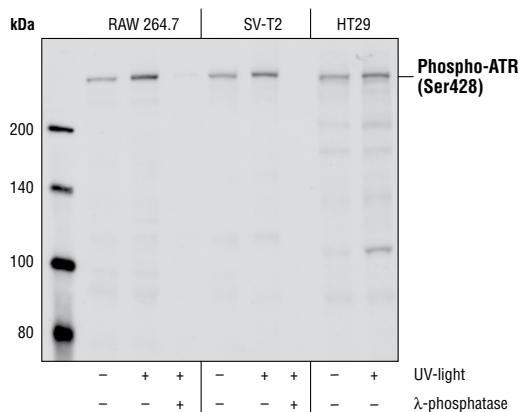
- (1) Kastan, M.B. and Lim, D.S. (2000) *Nat. Rev. Mol. Cell Biol.* 1, 179–186.
- (2) Abraham, R.T. *DNA Repair (Amst)* 3, 883–887.
- (3) Shechter, D. et al. *DNA Repair (Amst)* 3, 901–908.
- (4) Shieh, S.Y. et al. (1997) *Cell* 91, 325–334.
- (5) Tibbetts, R.S. et al. (1999) *Genes Dev.* 13, 152–157.
- (6) Martinho, R.G. et al. (1998) *EMBO J.* 17, 7239–1749.
- (7) Matsuoka, S. et al. (2000) *Proc. Natl. Acad. Sci. USA* 97, 10389–10394.
- (8) Melchionna, R. et al. (2000) *Nat. Cell Biol.* 2, 762–765.
- (9) Ahn, J.Y. et al. (2000) *Cancer Res.* 60, 5934–5936.
- (10) Rogakou, E.P. et al. (1998) *J. Biol. Chem.* 273, 5858–5868.
- (11) Burma, S. et al. (2001) *J. Biol. Chem.* 276, 42462–42467.
- (12) Rogakou, E.P. et al. (1999) *J. Cell Biol.* 146, 905–916.



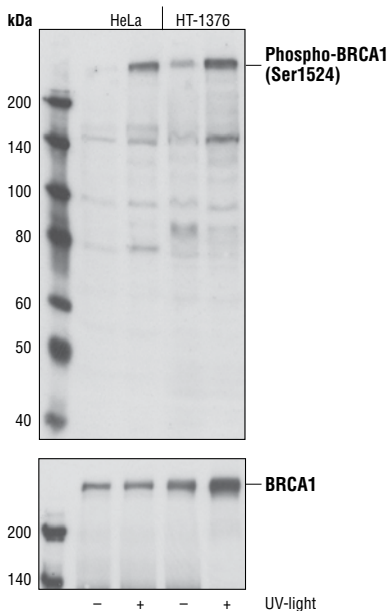
Selected rabbit monoclonal antibodies are produced under license (granting certain rights including those under U. S. Patent No. 5,675,063 and/or U.S.S.N. 11/476,277) from Epitomics, Inc. U.S.S.N. 11/476,277) from Epitomics, Inc.

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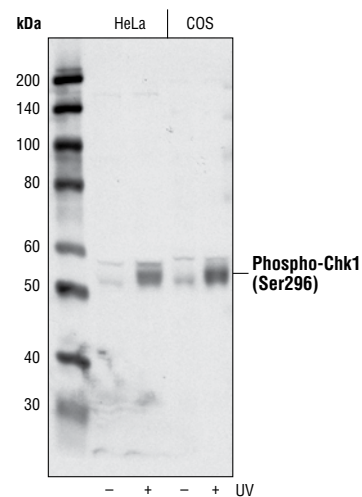
Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine
 Dg—dog Pg—pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



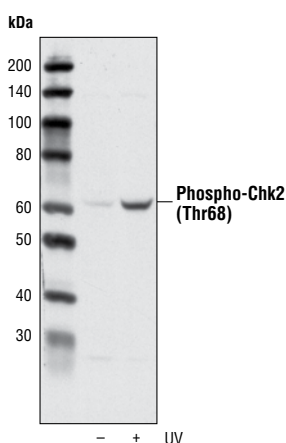
Western blot analysis of Raw264.7, SV-T2 and HT-29 cells that were untreated or UV-treated (50 mJ, 30 min), using **Phospho-ATR (Ser428) Antibody #2853**. λ phosphatase was used to demonstrate the phospho-specificity of the antibody.



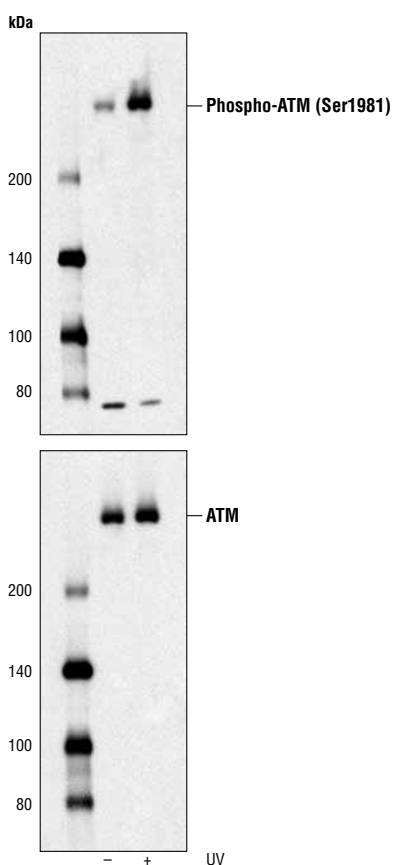
Western blot analysis of extracts from HeLa cells and HT-1376 cells, untreated and UV-treated (50 mJ/cm², 30 min), using **Phospho-BRCA1 (Ser1524) Antibody #9009** (upper) and **BRCA1 Antibody #9010** (lower).



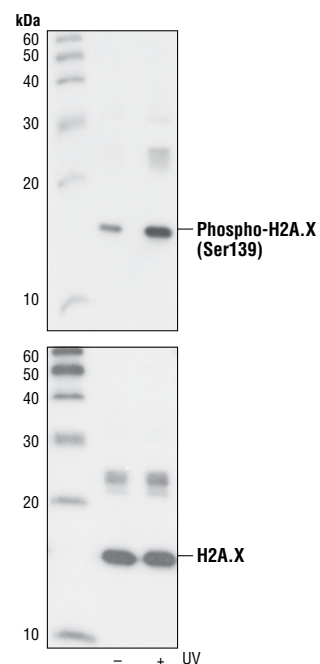
Western blot analysis of extracts from 293 and MvLu cells treated with UV or hydroxyurea (HU) as indicated, using **Phospho-Chk1 (Ser296) Antibody #2349**.



Western blot analysis of extracts from 293 cells, untreated, UV-treated or doxorubicin-treated (0.5 μM), using **Phospho-Chk2 (Thr68) Antibody #2661**.



Western blot analysis of extracts from 293 cells, untreated or UV-treated (100 mJ, 4 hr recovery), using **Phospho-ATM (Ser1981) (D6H9) Rabbit mAb #5883** (upper) or **ATM (D2E2) Rabbit mAb #2873** (lower).



Western blot analysis of extracts from untreated or UV-treated 293 cells, using **Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb # 9718** (upper) or **Histone H2A.X Antibody #2595** (lower).

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.