Store at -20°C

#9947

DNA Damage Antibody Sampler Kit

☑ 1 Kit
(7 x 20 µl)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-ATM (Ser1981) (D6H9) Rabbit mAb	5883	20 µl	350 kDa	Rabbit IgG
Phospho-ATR (Ser428) Antibody	2853	20 µl	300 kDa	Rabbit IgG
Phospho-BRCA1 (Ser1524) Antibody	9009	20 µl	220 kDa	Rabbit IgG
Phospho-Chk1 (Ser345) (133D3) Rabbit mAb	2348	20 µl	56 kDa	Rabbit IgG
Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb	9718	20 µl	15 kDa	Rabbit IgG
Phospho-Chk2 (Thr68) (C13C1) Rabbit mAb	2197	20 µl	62 kDa	Rabbit IgG
Phospho-p53 (Ser15) (16G8) Mouse mAb	9286	20 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.

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 validation data and a complete listing of recommended companion products.

Description: This kit provides an economical means to analyze major signaling checkpoints in response to DNA damage. The kit includes enough antibody to perform two western blot experiments with each primary 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 peptide and are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with recombinant human proteins or synthetic peptides.

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.

U.S. Patent No. 5,675,063

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 Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.