Kit Includes	Quantity	Applications	Reactivity	MW (kDa)	Isotype
Phospho-ATR (Ser428) Antibody #2853	40 µl	W	H M R Mk	300	Rabbit
Phospho-BRCA1 (Ser1524) Antibody #9009	40 µl	W	H	220	Rabbit
Phospho-Chk1 (Ser345) (133D3) Rabbit mAb #2348	40 µl	W IF-IC F	H M R Mk	56	Rabbit IgG
Phospho-Chk2 (Thr68) Antibody #2661	40 µl	W IP IF-IC F	H Mk	62	Rabbit
Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb #9718	40 µl	W IHC-P IF-IC F	H M R Mk	15	Rabbit IgG
Phospho-p53 (Ser15) (16G8) Mouse mAb #9286	40 µl	W IF-IC F	H	53	Mouse IgG1
Phospho-ATM (Ser1981) (D6H9) Rabbit mAb #5883	40 µl	W	H (Mk) (B) (Pg) (Hr)	350	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody #7074	100 µl				Goat
Anti-mouse IgG, HRP-linked Antibody #7076	100 µl				Horse

Applications Key: W=Western Blotting IP=Immunoprecipitation IHC-P=Immunohistochemistry (Paraffin) IF-IC=Immunofluorescence (Immunocytochemistry) F=Flow Cytometry

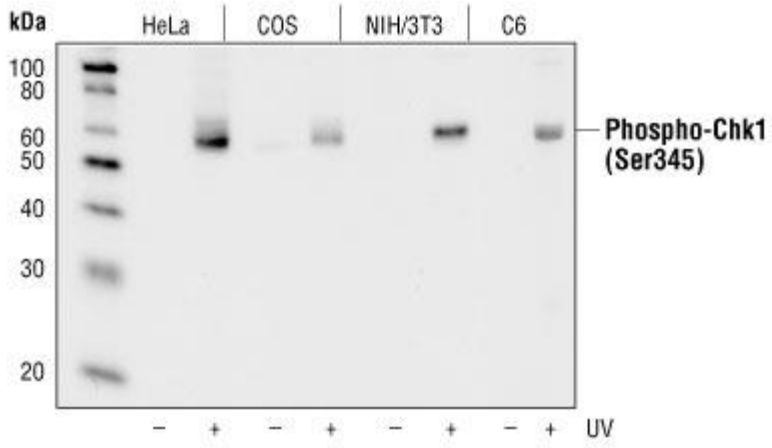
Reactivity Key: H=Human M=Mouse R=Rat Mk=Monkey B=Bovine Pg=Pig Hr=Horse

Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Specificity / Sensitivity

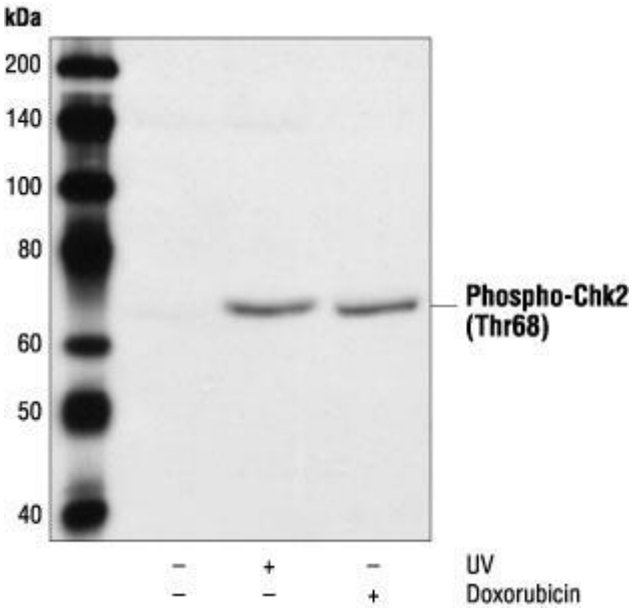
All antibodies in the DNA Damage Antibody Sampler Kit recognize their targets proteins only when modified at the indicated site.

Western Blotting



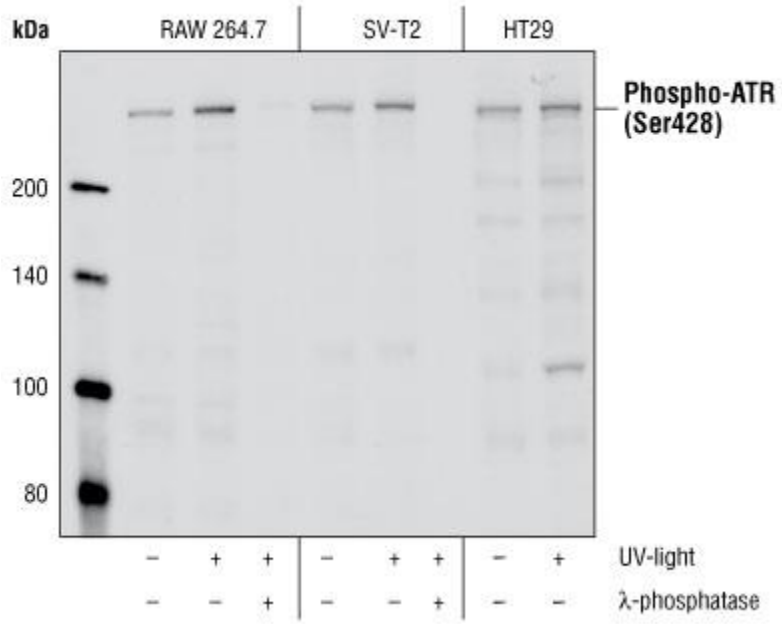
Western blot analysis of extracts from HeLa, COS, NIH/3T3 and C6 cells, untreated or UV-treated, using Phospho-Chk1 (Ser345) (133D30) Rabbit mAb #2348.

Western Blotting



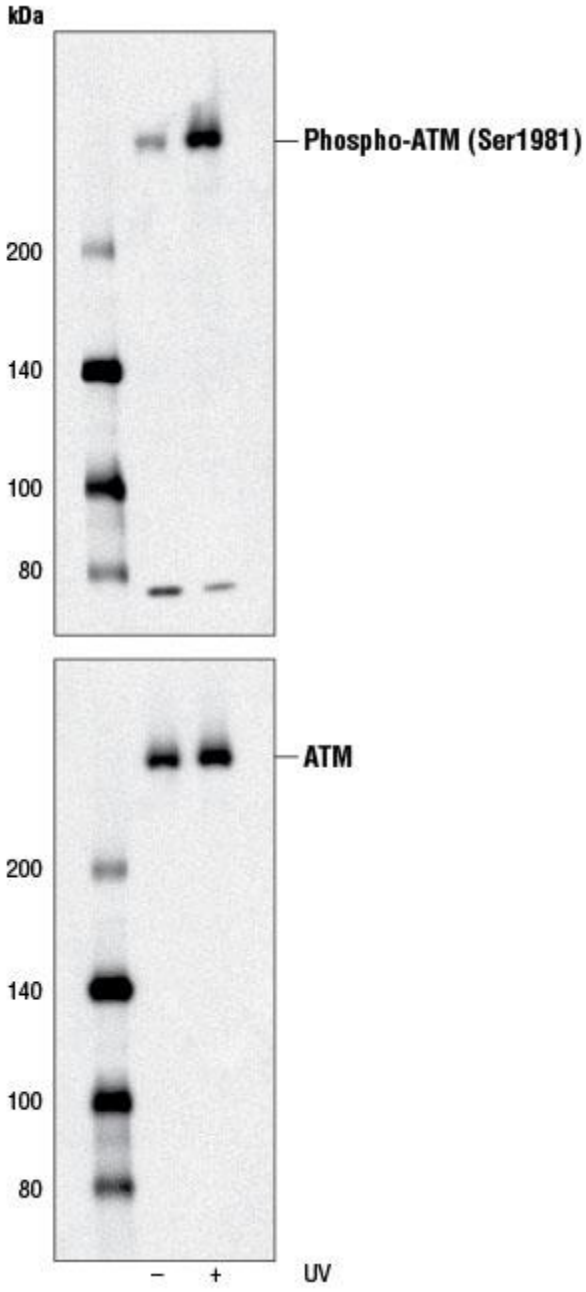
Western blot analysis of extracts from 293 cells, untreated, UV-treated or doxorubicin-treated (0.5 µM), using Phospho-Chk2 (Thr68) Antibody #2661.

Western Blotting



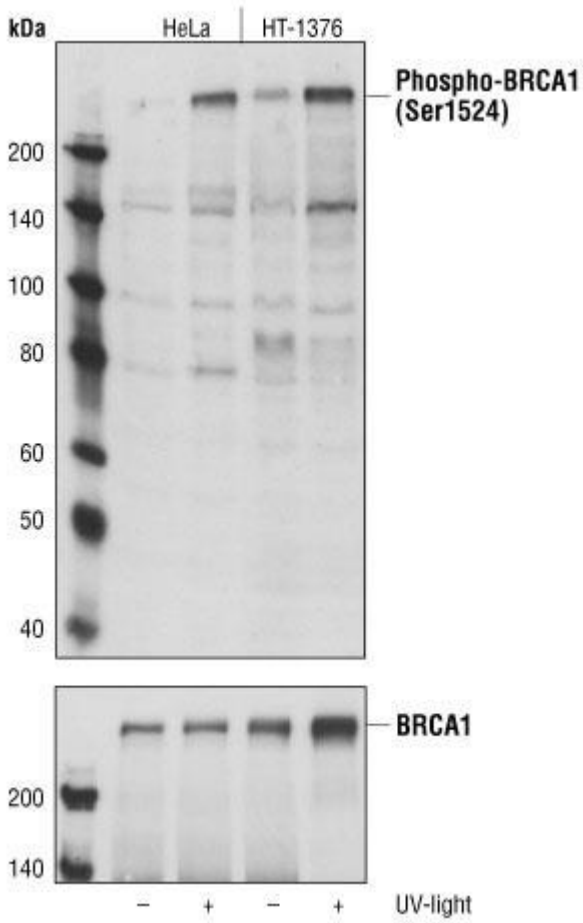
Western blot analysis of Raw264.7, SV-T2 and HT-29 cells, untreated or UV-treated (50 mJ, 30 min), using Phospho-ATR (Ser428) Antibody #2853. λ phosphatase was used to demonstrate the phospho-specificity of the antibody.

Western Blotting



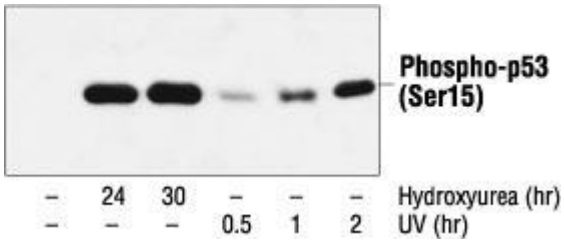
Western blot analysis of extracts from 293 cells, untreated or UV-treated (100 mJ, 4 hr recovery), using Phospho-ATM (Ser1981) (D6H9) Rabbit mAb #5883 (upper) or ATM (D2E2) Rabbit mAb #2873 (lower).

Western Blotting



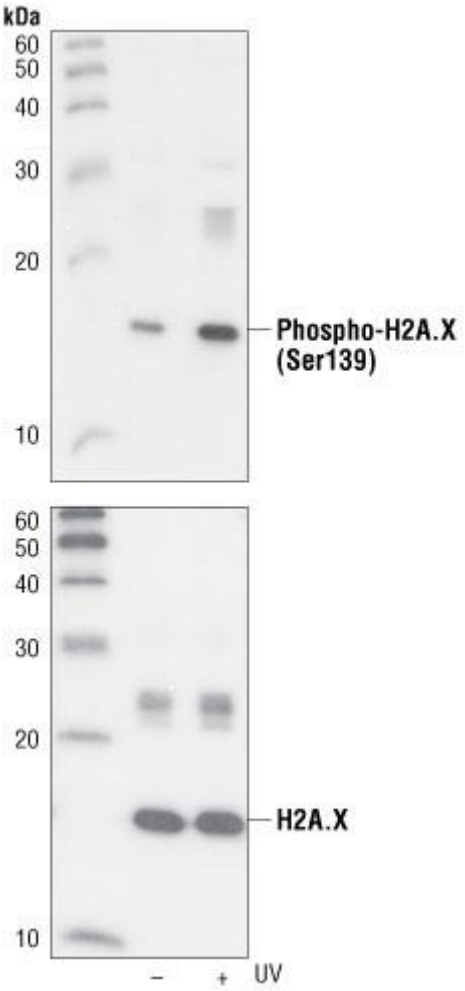
Western blot analysis of extracts from HeLa cells and HT-1376 cells, untreated and UV-treated (50 mJ/cm2, 30 min), using Phospho-BRCA1 (Ser1524) Antibody #9009 (upper) and BRCA1 Antibody #9010 (lower).

Western Blotting



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

Western Blotting



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).

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.

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; 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; Ser345 of Chk1; or Ser15 of human p53.

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).

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