Store at -20°C

#3428

Phospho-53BP1 (Thr543) Antibody

Cell Signaling TECHNOLOGY®

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

Entrez-Gene ID #7158
Swiss-Prot Acc. #Q12888

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, (Mk)	450 kDa	Rabbit**

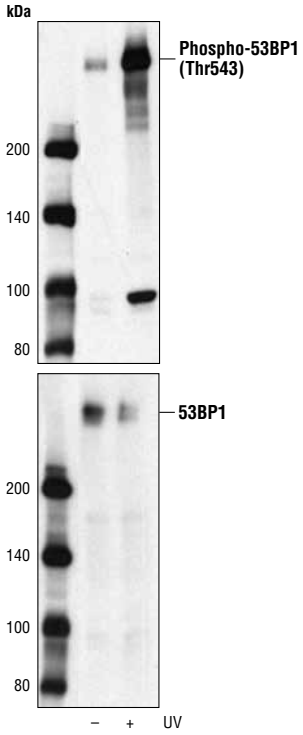
Background: p53-binding protein 1 (53BP1) was originally identified as a p53 binding partner that could enhance the transcriptional activity of p53 (1,2). 53BP1 consists of two BRCA1 carboxy terminal (BRCT) domains that allow for binding to p53 and a separate domain responsible for binding to phosphorylated histone H2A.X (3). 53BP1 rapidly translocates to nuclear foci following treatment of cells with ionizing radiation (IR) or radiomimetic agents that cause DNA double strand breaks (DSBs) (4,5). Because of this localization to DSBs and homology to the yeast protein Rad9, a role for 53BP1 in DSB repair has been proposed. Recruitment of 53BP1 to sites of DNA damage has been demonstrated to be independent of ATM, NBS1, and DNA-PK (4) and retention of 53BP1 at DNA breaks requires phosphorylated H2A.X (6). In cells lacking 53BP1, phosphorylation of ATM substrates is reduced, suggesting that 53BP1 is upstream of ATM (7). In response to IR, phosphorylation of 53BP1 at serines 6, 25, 29, and 784 by ATM has been demonstrated, but phosphorylation at these sites is not required for localization of 53BP1 to sites of DSBs (6).

Threonine 543 of 53BP1 has been shown to be phosphorylated in an ATM/ATR-dependent manner in response to DNA damage (8,9).

Phospho-53BP1 (Thr543) Antibody is directed at a site that was identified at Cell Signaling Technology (CST) using PhosphoScan®, CST's LC-MS/MS platform for modification site discovery. Phosphorylation at Thr543 was discovered using an ATM/ATR substrate antibody and was shown to be induced by UV treatment. Please visit PhosphoSitePlus™, CST's modification site knowledgebase, at www.phosphosite.org for more information.

Specificity/Sensitivity: Phospho-53BP1 (Thr543) Antibody detects endogenous levels of 53BP1 protein only when phosphorylated at Thr543.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr543 of human 53BP1. Antibodies are purified using protein A and peptide affinity chromatography.



Western blot analysis of extracts from M059K cells, untreated or UV-treated, using Phospho-53BP1 (Thr543) Antibody (upper) or 53BP1 Antibody #4937 (lower).

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.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000

For application specific protocols please see the web page for this product at www.cellsignal.com.

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

Background References:

(1) Iwabuchi, K. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91, 6098–6102.

(2) Iwabuchi, K. et al. (1998) *J. Biol. Chem.* 273, 26061–26068.

(3) Mochan, T.A. et al. (2004) *DNA Repair (Amst)* 3, 945–952.

(4) Schultz, L.B. et al. (2000) *J. Cell Biol.* 151, 1381–1390.

(5) Anderson, L. et al. (2001) *Mol. Cell. Biol.* 21, 1719–1729.

(6) Ward, I.M. et al. (2003) *J. Biol. Chem.* 278, 19579–19582.

(7) DiTullio, R.A. et al. (2002) *Nat. Cell Biol.* 4, 998–1002.

(8) Stokes, M.P. et al. (2007) *Proc. Natl. Acad. Sci. USA* 104, 19855–19860.

(9) Matsuoka, S. et al. (2007) *Science* 316, 1160–1166.

IMPORTANT: 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.

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 **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.