#2675 Store at –20°C

Phospho-53BP1 (Ser1778) Antibody

Cell Signaling TECHNOLOGY®

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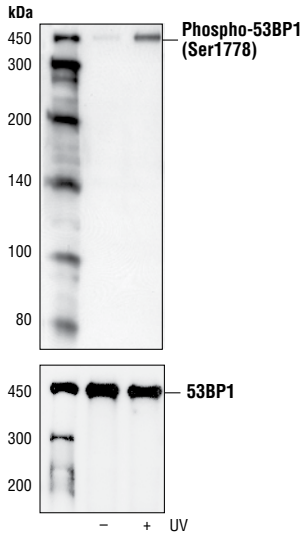
For Research Use Only. Not For Use In Diagnostic Procedures.

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

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IF-IC, F Endogenous	H, Mk	450 kDa	Rabbit**

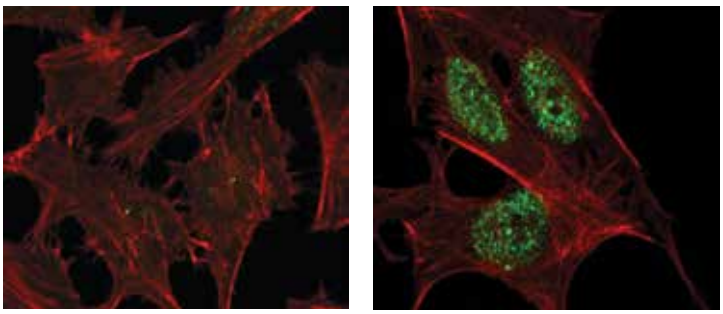
Background: p53-binding protein 1 (53BP1) was originally identified as a p53 binding partner that could enhance the transcriptional activity of p53 (1,2). 53BP1 consists of two BRCA1 carboxy-terminal (BRCT) domains that allow for binding to p53 and a separate domain responsible for binding to phosphorylated 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). Within the first BRCT domain (amino acids 1714–1850), there exists a consensus ATM/ATR phosphorylation site, Ser1778. It is conceivable that phosphorylation of Ser1778 could therefore serve to regulate 53BP1-p53 binding.

Specificity/Sensitivity: Phospho-53BP1 (Ser1778) Antibody detects endogenous levels of 53BP1 only when phosphorylated at serine 1778.



Western blot analysis of extracts from 293 cells, untreated or UV-treated (50 mJ for 2 hours), using Phospho-53BP1 (Ser1778) Antibody (upper) or 53BP1 Antibody #4937 (lower).

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser1778 of human 53BP1. Antibodies are purified by protein A and peptide affinity chromatography.



Confocal immunofluorescent analysis of HeLa cells, untreated (left) or UV-treated (right), using Phospho-53BP1 (Ser1778) Antibody (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red).

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:

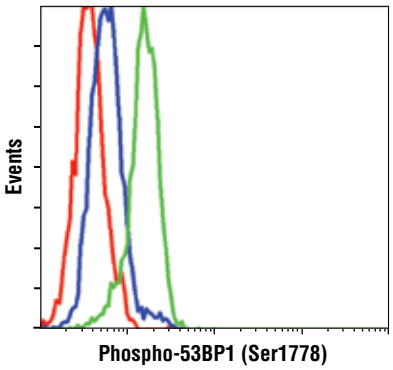
Western Blotting	1:1000
Immunofluorescence (IF-IC)	1:100
Flow Cytometry	1:100

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 U S A* 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.



Flow cytometric analysis of HeLa cells, untreated (blue) or UV-treated (green), using Phospho-53BP1 (Ser1778) Antibody compared with a nonspecific negative control antibody.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% 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.