

53BP1 Antibody

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

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

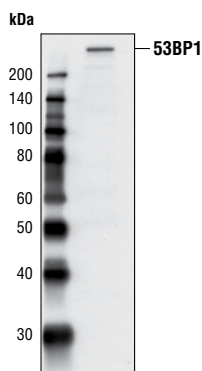
Background: p53-binding protein 1 (53BP1) was originally identified as a p53 binding partner that could enhance the transcriptional activity of p53 (1,2). 53BP1 consists of two BRCA1 carboxy terminal (BRCT) domains that allow for binding to p53 and a separate domain responsible for binding to phosphorylated 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).

Specificity/Sensitivity: 53BP1 Antibody detects endogenous levels of total 53BP1 protein independent of phosphorylation.

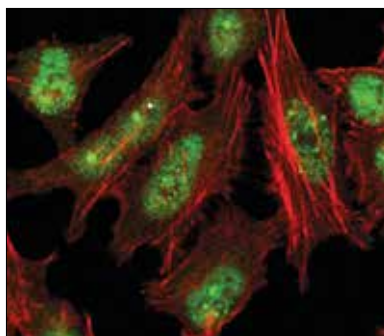
Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues near the center of human 53BP1. Antibodies are purified by protein A and peptide affinity chromatography.

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.



Western blot analysis of extracts from HT29 cells, using 53BP1 Antibody.



Confocal immunofluorescent analysis of HeLa cells using 53BP1 Antibody (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red).

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

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:

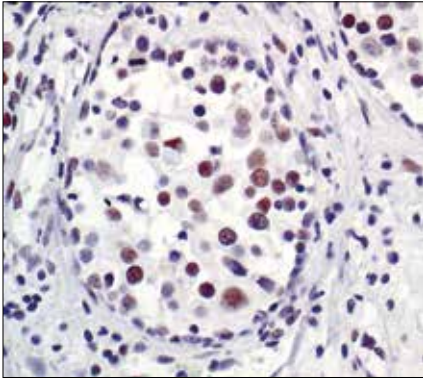
Western Blotting	1:1000
Immunohistochemistry (Paraffin)	1:100
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114
Immunofluorescence (IF-IC)	1:100

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

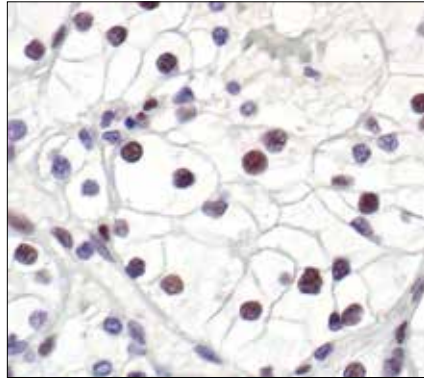
Please visit www.cellsignal.com for a complete listing of recommended companion products.

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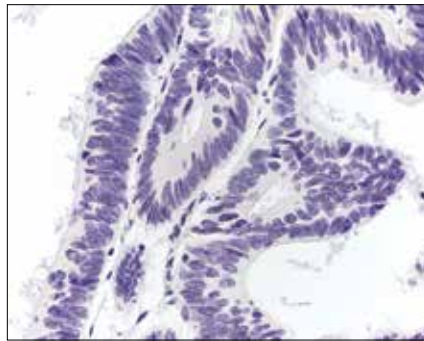
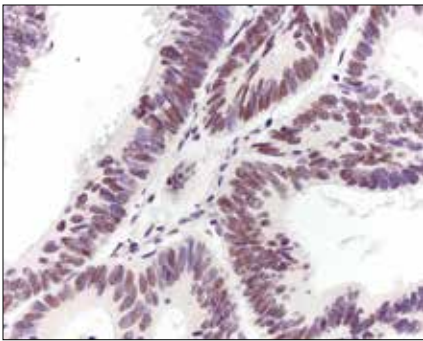
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma, showing nuclear localization, using 53BP1 Antibody.



Immunohistochemical analysis of paraffin-embedded human renal cell carcinoma, using 53BP1 Antibody.



Immunohistochemical analysis of paraffin-embedded human colon carcinoma, using 53BP1 Antibody in the presence of control peptide (left) or antigen-specific peptide (right).