

#2674 Store at -20°C

# Phospho-53BP1 (Ser25/29) Antibody

✓ 100 µl  
(10 Western mini-blot)



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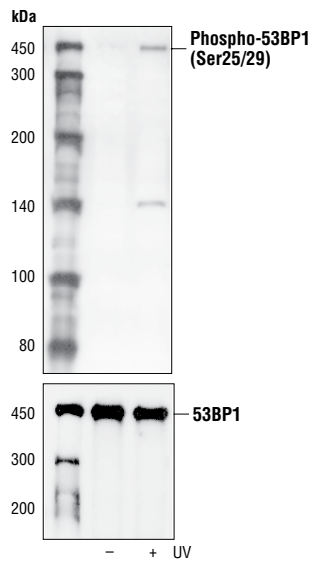
This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity	Molecular Wt.	Source
W	H	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 (BRC1) 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:** Phospho-53BP1 (Ser25/29) Antibody detects endogenous levels of 53BP1 only when phosphorylated at serine 25/29.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Ser25/29 of human 53BP1. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from 293 cells, untreated or UV-treated (50 µJ for 2 hours), using Phospho-53BP1 (Ser25/29) Antibody (upper) or 53BP1 Antibody #4937 (lower).

### 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.

**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.

**Recommended Antibody Dilutions:**  
Western Blotting 1:1000

**Companion Products:**  
53BP1 Antibody #4937  
p53 (1C12) Mouse mAb #2524  
Phospho-Histone H2A.X (Ser139) Antibody #2577  
Phospho-Chk2 (Thr68) Antibody #2661  
Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071  
Anti-rabbit IgG, HRP-linked Antibody #7074  
Prestained Protein Marker, Broad Range (Premixed Format) #7720  
Biotinylated Protein Ladder Detection Pack #7727  
20X LumiGLO® Reagent and 20X Peroxide #7003

**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 IC—Immunocytochemistry IF—Immunofluorescence F—Flow cytometry E—ELISA D—DELFIATM  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine All—all species expected  
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

## Western Immunoblotting Protocol (Primary Ab Incubation In BSA)

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.

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope®-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

**NOTE:** CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO® substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.