

#3001 Store at -20°C

Phospho-p95/NBS1 (Ser343) Antibody

✓ 100 µl
(10 Western mini-blots)



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

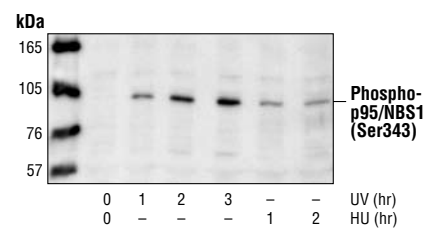
Applications W, E-P	Species Cross-Reactivity* H, M, Mi	Molecular Wt. 95 kDa	Source Rabbit
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Background: The Nijmegen breakage syndrome (NBS) is characterized by defects in cell cycle checkpoints, growth retardation, an increased propensity for cancer and sensitivity to ionizing radiation (1). Repair of DNA double-strand breaks by radiation is dependent on a multifunctional complex containing Rad50, Mre11 and the NBS1 gene product p95/NBS1 (also called p95 or nibrin) (2). p95/NBS1 is a protein with a forkhead-associated domain and a carboxy-terminal repeat frequently found in cell cycle regulatory and DNA repair proteins (1,3). The overlap between clinical and cellular phenotypes in ataxia telangiectasia (AT) and NBS suggests that AT-mutated (ATM) and p95/NBS1 function in the same biochemical pathway. ATM interacts with and phosphorylates p95/NBS1 at Ser343 after exposure to ionizing radiation (4–7).

Specificity/Sensitivity: Phospho-p95/NBS1 (Ser343) Antibody detects endogenous levels of p95/NBS1 only when phosphorylated at serine 343.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Ser343 of human p95/NBS1. Antibodies are purified by protein A and peptide affinity chromatography.

Selected Application References:
Lukas, C. et al. (2003) Distinct spatiotemporal dynamics of mammalian checkpoint regulators induced by DNA damage. *Nat. Cell Biol.* 5, 255–260. Applications: IC-IF, W.



Western blot analysis of extracts from Mv1Lu cells treated with UV or hydroxyurea (HU) for the indicated times, using Phospho-p95/NBS1 (Ser343) Antibody.

Background References:

- (1) Varon, R. et al. (1998) *Cell* 93, 467–476.
- (2) Carney, J.P. et al. (1998) *Cell* 93, 477–486.
- (3) Durocher, D. et al. (1999) *Mol. Cell* 4, 387–394.
- (4) Gatei, M. et al. (2000) *Nat. Genet.* 25, 115–119.
- (5) Lim, D.S. et al. (2000) *Nature* 404, 613–617.
- (6) Wu, X. et al. (2000) *Nature* 405, 477–482.
- (7) Zhao, S. et al. (2000) *Nature* 405, 473–477.

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.

Recommended Antibody Dilutions:
Western blotting 1:1000
ELISA-Peptide 1:200

Companion Products:
Phospho-Chk1 (Ser345) Antibody #2341
Phospho-Chk2 (Thr68) Antibody #2661
Phospho-p53 (Ser15) Antibody #9284
Phospho-p53 (Ser15) (16G8) Mouse mAb #9286
Phospho-p53 (Ser20) Antibody #9287
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% w/v 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 Antibody 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 cm²) 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.

ELISA-Peptide Assay Protocol

A Solutions and Reagents

- 1. Carbonate Buffer:** 15 mM Na₂CO₃, 35 mM NaHCO₃, 0.2 g/L NaN₃ (pH 9.6). Use 1 μM synthetic peptide in carbonate buffer.
- 2. 10X Phosphate Buffered Saline (PBS):** To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na₂HPO₄) and 2.4 g potassium phosphate, monobasic (KH₂PO₄) to 1 L dH₂O. Adjust pH to 7.4.
- 3. Wash Buffer:** 1X PBS containing 0.05% Tween-20 (PBST)
- 4. Blocking Buffer:** 10 mg/ml bovine serum albumin (BSA) in PBST
- 5. Antibody Dilution Buffer:** 3% BSA in PBST
- 6. DELFIA® Europium-labeled Anti-mouse IgG** for mouse primary antibodies or Anti-rabbit IgG (PerkinElmer Life Sciences #AD0124) for rabbit primary antibodies.
- 7. DELFIA® Enhancement Solution** (PerkinElmer Life Sciences #1244-105)

(DELFLIA® is a registered trademark of PerkinElmer, Inc.)

B Protocol

- 1.** Coat the wells of a 96-well microtiter plate with 100 μl of 1 μM synthetic peptide in carbonate buffer by incubating overnight at 4°C or for 2 to 6 hours at 37°C. If the peptide does not bind or absorb, try other buffers in the pH 4–8 range.
- 2.** Wash plate three times 200 μl/well with wash buffer.
- 3.** Block plate with 200 μl/well blocking buffer for 1 hour at 37°C. Wash plate three times with wash buffer. (May leave dry plate at 4°C for 1–2 months if desired.)
- 4.** Prepare appropriate dilution of primary antibody with antibody dilution buffer. Add 100 μl to wells and incubate at 37°C for 1 hour.
- 5.** Wash three times with wash buffer.
- 6.** Add 67 ng/well DELFLIA® Europium-labeled Anti-mouse IgG, diluted in 100 μl/well antibody dilution buffer. Incubate at 37°C for 30 minutes.
- 7.** Wash five times with wash buffer.
- 8.** Add 100 μl enhancement solution and incubate at 37°C for 15 minutes. Read plate at 615 nm with an appropriate time-resolved plate reader.