

# Product Datasheet

## Nbs1 Antibody NB100-143

Unit Size: 0.05 ml

Store at 4C. Do not freeze.

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**NB100-143**

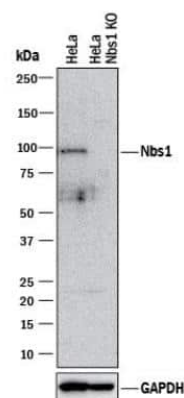
Nbs1 Antibody

Product Information	
Unit Size	0.05 ml
Concentration	This product is unpurified. The exact concentration of antibody is not quantifiable.
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Unpurified
Buffer	Whole antisera
Target Molecular Weight	85 kDa
Product Description	
Host	Rabbit
Gene ID	4683
Gene Symbol	NBN
Species	Human, Mouse, Hamster, Mammal, Primate
Reactivity Notes	Human and Mouse reactivity reported in multiple pieces of scientific literature. Sumatran orangutan ( <i>Pongo abelii</i> ), white-cheeked gibbon ( <i>Nomascus leucogenys</i> ), and rhesus macaque ( <i>Macaca mulatta</i> ) reactivity reported in scientific literature (PMID: 27512903). Hamster reactivity reported in scientific literature (PMID: 23255801). Potorous tridactylus reactivity reported in scientific literature (PMID: 24064949). African green monkey reactivity reported in scientific literature (PMID: 24064949).
Immunogen	Nbs1 Antibody was made to a partial length human NBS1 protein [Swiss-Prot: O60934].
Product Application Details	
Applications	Western Blot, Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, In-situ Hybridization, SDS-Page, Chromatin Immunoprecipitation (ChIP), Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 1:1000. Use reported in multiple pieces of scientific literature, Chromatin Immunoprecipitation reported in multiple pieces of scientific literature, Flow Cytometry reported in multiple pieces of scientific literature, ELISA, Immunohistochemistry 1:100 - 1:200, Immunocytochemistry/ Immunofluorescence 1:50-1:200. Use reported in multiple pieces of scientific literature, Immunoprecipitation 3 ul. Use reported in multiple pieces of scientific literature, Immunohistochemistry-Paraffin 1:100 - 1:200, Immunoblotting, In-situ Hybridization reported in scientific literature (PMID 26415217), SDS-Page, Chromatin Immunoprecipitation (ChIP), Knockout Validated, Knockdown Validated
Application Notes	By Western blot, this NBS1 antibody recognizes a band at 95 kDa, representing NBS1. By ICC/IF, this antibody has been used with methanol-fixed IMR90 primary human fibroblasts. IP tests have been done with 3-4 x 10 <sup>6</sup> cells.

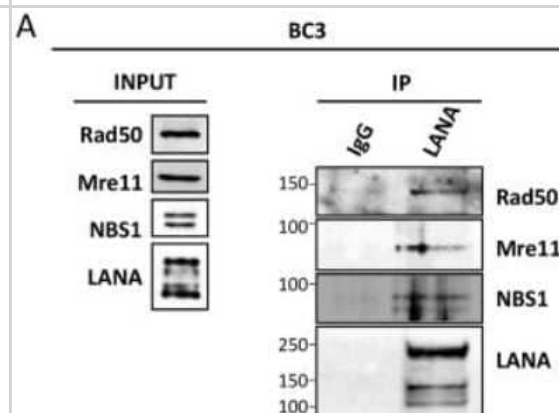


## Images

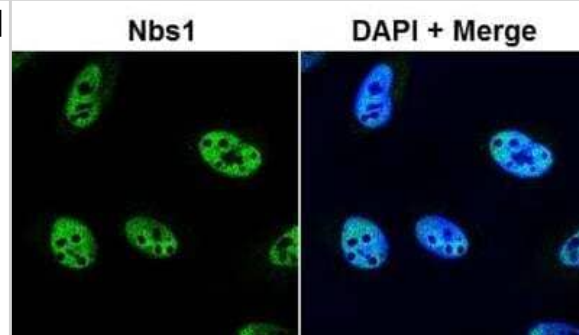
**Knockout Validated: Nbs1 Antibody [NB100-143]** - Lysates of HeLa human cervical epithelial carcinoma parental cell line and Nbs1 knockout (KO) HeLa cell line. PVDF membrane was probed with 1:1000 of Rabbit Anti-Human Polyclonal [NB100-143] followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (HAF008). Specific band was detected for Nbs1 at approximately 95 kDa (as indicated) in the parental HeLa cell line, but is not detectable in the knockout HeLa cell line. This experiment was conducted under reducing conditions.



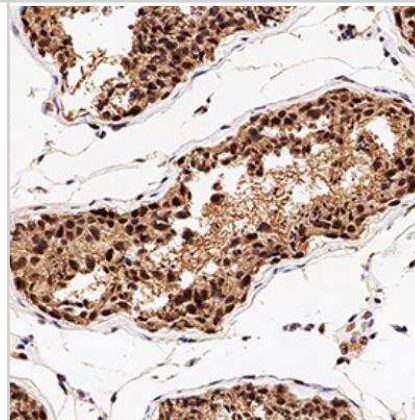
**Western Blot: Nbs1 Antibody [NB100-143]** - Western Blot with Nbs1 Antibody [NB100-143]. KSHV LANA recruits MRN (Mre11-Rad50-NBS1) complex. Co-immunoprecipitation of endogenous LANA and MRN proteins in BC3 cells. Cells were lysed using TBS-T buffer and the cell lysate was incubated with benzonase. After centrifugation, supernatant was incubated overnight with anti-LANA or IgG-control beads. The precipitated complexes were analyzed for the presence of endogenous Rad50, Mre11 and NBS1 by SDS-PAGE and immunoblotting. Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.ppat.1006335>), licensed under a CC-BY license.



**Immunocytochemistry/Immunofluorescence: Nbs1 Antibody [NB100-143]** - ICC/IF analysis of Nbs1 in HeLa cells with Nbs1 Antibody [NB100-143]. Image from verified customer review.



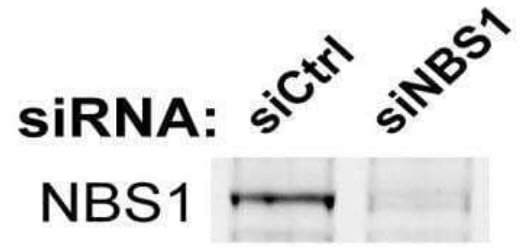
**Immunohistochemistry-Paraffin: Nbs1 Antibody [NB100-143]** - FFPE human testis using Nbs1 antibody [NB100-143] at 1:200 on a Bond Rx autostainer (Leica Biosystems). The assay involved 20 minutes of heat induced antigen retrieval (HIER) using 10 mM sodium citrate buffer (pH 6.0) and endogenous peroxidase quenching with peroxide block. The sections were incubated with primary antibody for 30 minutes and Bond Polymer Refine Detection (Leica Biosystems) with DAB was used for signal development followed by counterstaining with hematoxylin. Whole slide scanning and capturing of representative images was performed using Aperio AT2 (Leica Biosystems). Staining was performed by Histowiz.



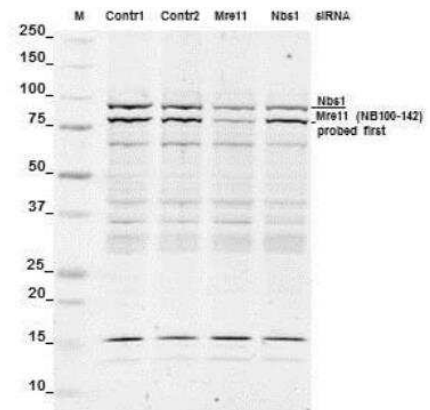
Western Blot: Nbs1 Antibody [NB100-143] - Analysis of HeLa whole cell lysate [NB800-PC1] using rabbit polyclonal NBS1 antibody [NB100-143]. Observed Molecular Weight at ~95 kDa.



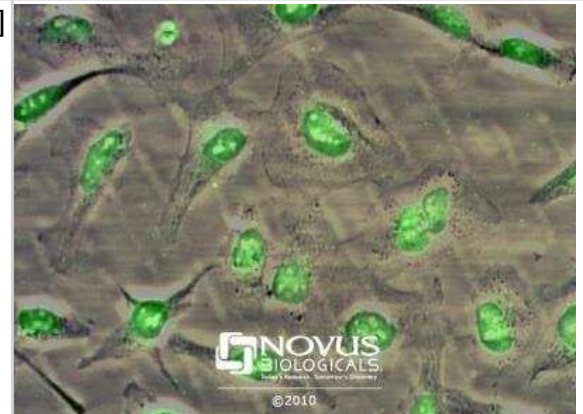
Western Blot: Nbs1 Antibody [NB100-143] - Detection of NBS1 in Human Bone Osteosarcoma Epithelial Cells (U2OS whole cell lysate) using Nbs1 Antibody [NB100-143] at a dilution of 1:1000. Specificity was confirmed using a siRNA anti-NBS1 (targeting sequence: GGAAGAAACGUGAACUCAA). Image provided by Sebastien Britton of the Institute of Pharmacology and Structural Biology.



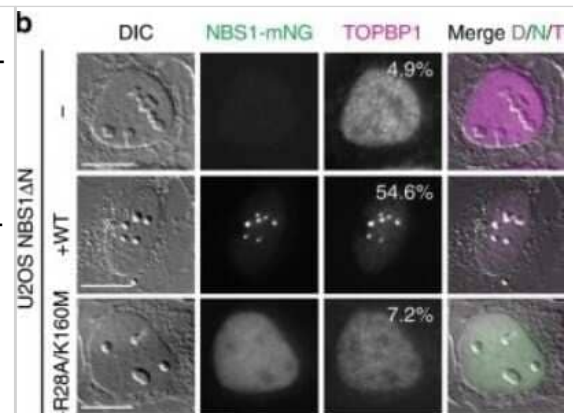
Western Blot: Nbs1 Antibody [NB100-143] - Western Blot with Nbs1 Antibody [NB100-143]. TIG-1 human primary fibroblasts, whole cell lysate (30 ug). Image from verified customer review.



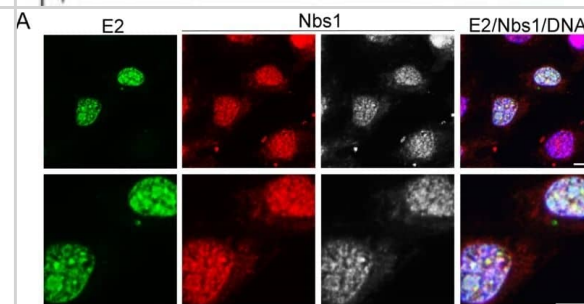
Immunocytochemistry/Immunofluorescence: Nbs1 Antibody [NB100-143] - Staining of HeLa cells using Nbs1 Antibody [NB100-143].



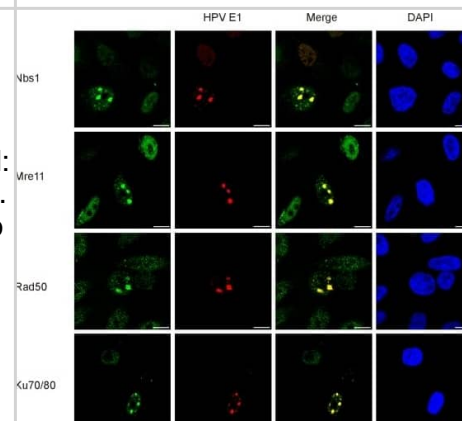
Knockdown Validated: Nbs1 Antibody [NB100-143] - TOPBP1 recruitment in response to rDNA breaks. TOPBP1 localization 2 h after I-Ppo1 transfection in NBS1-delta-N cells and NBS1-delta-N cells complemented with wild-type and mutant NBS1-mNG (percentage of cells with > 2 TOPBP1 caps are indicated; one of two experiments is shown). Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31913317/>) licensed under a CC-BY license.



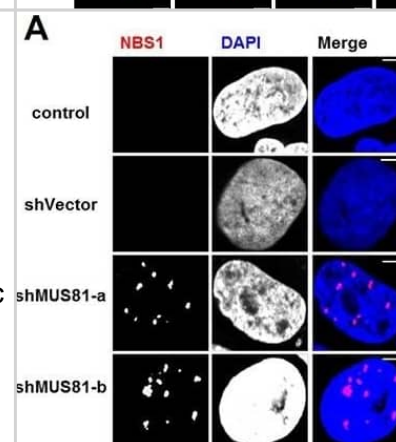
HPV16 E2 partially colocalizes with the MRN complex proteins Mre11 and Nbs1. C33a cells were transfected with an HPV16 E2 expression plasmid, fixed in ice-cold methanol, and stained with an E2-specific antibody (green). DNA was stained with Hoechst 33342 (blue). (A) Endogenous Nbs1 was detected with an Nbs1-specific antibody (red/gray). (B) Endogenous Mre11 was detected with an Mre11-specific rabbit antibody (red/gray). Bar, 5  $\mu$ m. Digital zoom is shown at the bottom.



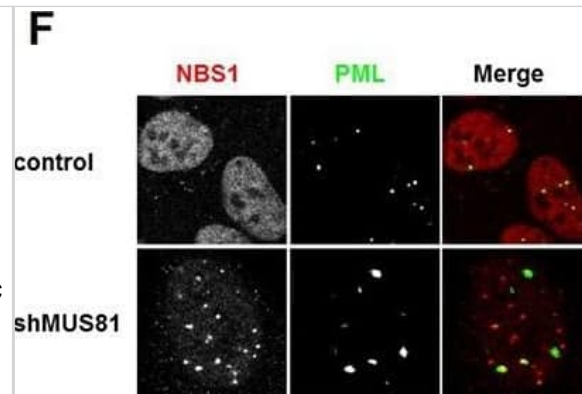
Immunocytochemistry/ Immunofluorescence: Nbs1 Antibody [NB100-143] - Replication centers of the integrated HPV recruit Mre11-Nbs1-Rad50 complex & Ku70/80 heterodimer. HeLa cells were transfected & analyzed as described previously. Co-immunostaining of HPV18 E1 (Alexa Fluor 568, second column) & the following proteins are presented: Mre11, Nbs1, Rad50, & Ku70/80 proteins (Alexa Fluor 488, first column). The localizations of the E1 & the respective DNA repair proteins are also shown in the third column as a merged image & DAPI stained nuclei are presented in the fourth column. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/19390600/>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



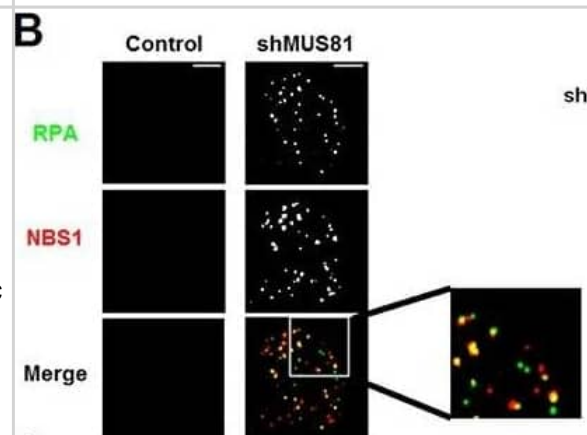
Immunocytochemistry/ Immunofluorescence: Nbs1 Antibody [NB100-143] - Loss of MUS81 activates DNA damage responseA. MUS81 depleted U2OS cells accumulate NBS1 foci defining sites of DNA damage. B. Partial co-localization of NBS1 foci & RPA foci in MUS81-depleted cells. C. Co-localization of RPA foci to ssDNA regions. Cells were labelled with BrdU for 3 days concomitant with MUS81 depletion. BrdU was detected at ssDNA regions by immunofluorescence. D. NBS1 foci arise in cells that are both positive & negative for Cyclin A expression. Scale bars, 5  $\mu$ m. E. NBS1 foci form largely at non-telomeric loci. F. Partial co-localization of NBS1 foci with PML nuclear bodies. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/26415217/>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



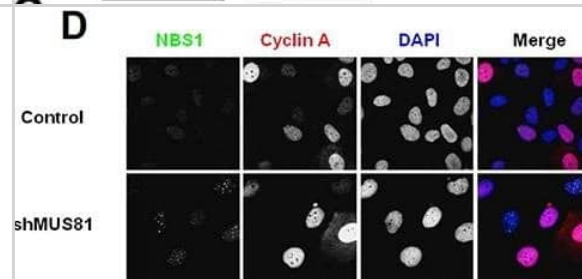
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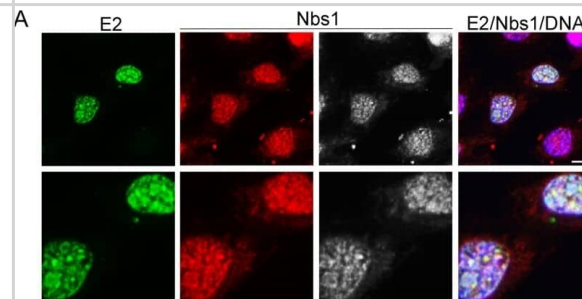
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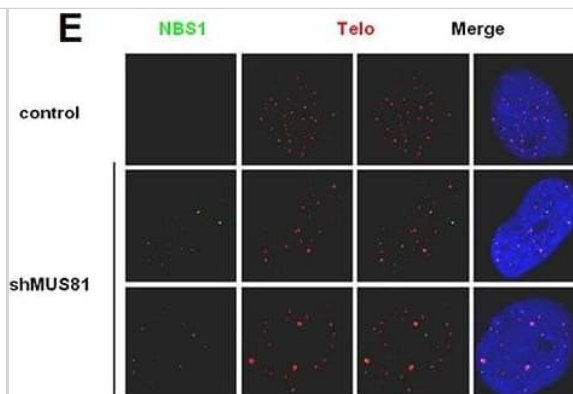
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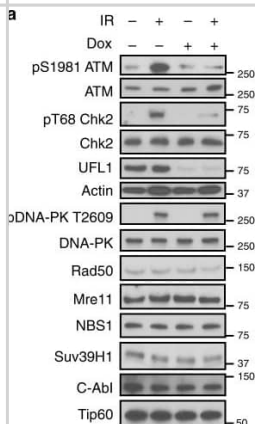
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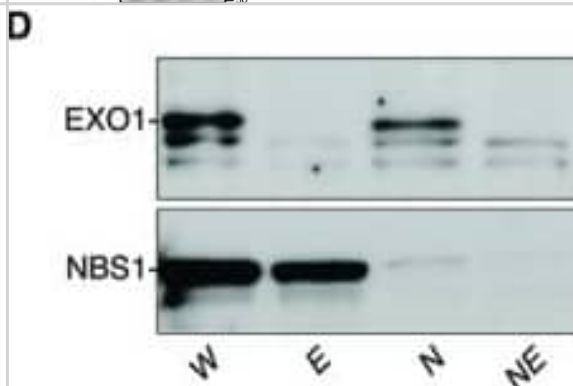
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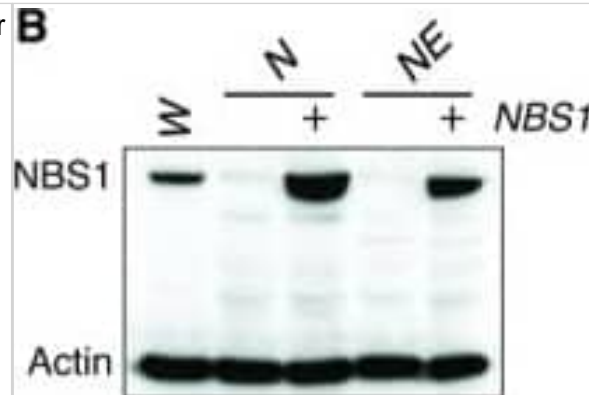
Western Blot: Nbs1 Antibody [NB100-143] - UFL1 regulates the ATM signaling. a U2OS cells expressing UFL1 tet-on shRNA1 were irradiated with 2 Gy IR. Thirty minutes later, cells were lysed & blotted with indicated antibodies. b Representative picture of  $\gamma$ H2AX, 53BP1, & BRCA1 foci in control (Dox-) & UFL1 knockdown (Dox+) U2OS cells 1 h after 0.5 Gy treatment. Scale bars, 10  $\mu$ m. c-e Quantification of intensities of  $\gamma$ H2AX, 53BP1, & BRCA1 foci in control (Dox-) & UFL1 knockdown (Dox+) U2OS cells. Data presented as mean  $\pm$  SD of n = 50 cells. \*\*p < 0.01 by Student's t-test. Source data are provided as a Source Data file Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30886146>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



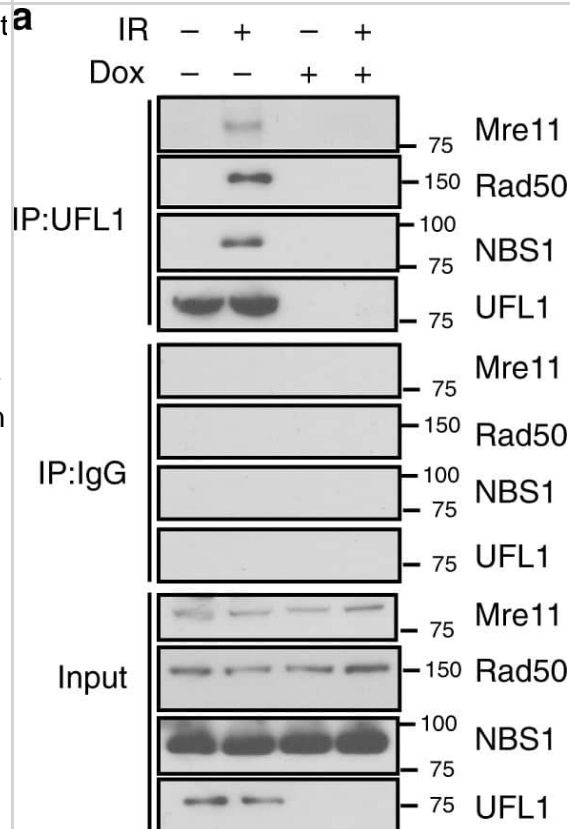
Western Blot: Nbs1 Antibody [NB100-143] - Deletion of Exo1 leads to embryonic lethality in hypomorphic Nbs1 mice. (A) Graph of expected & observed live born pups from double heterozygous breedings based on normal Mendelian inheritance (n = 93). For brevity, the primary genotypes are abbreviated as follows: wild type = W, Nbs1 $\Delta$ B/ $\Delta$ B = N, Exo1 $^{-/-}$  = E, Nbs1 $\Delta$ B/ $\Delta$ BExo1 $^{-/-}$  = NE. Statistical analysis was performed using an unpaired t-test. \*\*\*P < 0.0001 & n.s. = not significant. (B) Graph of expected & observed E14.5 embryos from double heterozygous breedings based on normal Mendelian inheritance (n = 89). (C) Representative images of E14.5 embryos of the indicated genotype. (D) Western blotting of NBS1 & EXO1 from embryonic fibroblast cultures derived from E14.5 embryos. Image collected & cropped by CiteAb from the following publication (<https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/gkv691>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Nbs1 Antibody [NB100-143] - EXO1 influences DNA repair & DNA replication in Nbs1 mutants. (A) Schematic illustration of the SA-GFP based SSA assay. (B) Western blotting of cells transfected with or without a vector expressing human NBS1. The genotypes are abbreviated throughout the figure as follows: wild type = W, Nbs1 $\Delta$ B/ $\Delta$ B = N, Exo1 $^{-/-}$  = E, and Nbs1 $\Delta$ B/ $\Delta$ B Exo1 $^{-/-}$  = NE. (C) Quantification of SSA mediated repair plotted as the percentage of GFP positive cells. Values for NE are corrected for the reduced percentage of cells in S/G2 determined by BrdU & PI staining (mean 58% compared to a mean of 73% in the other genotypes). The fold rescue with NBS1 expression is the same in N & NE (2.2 fold). (D) Measurement of replication tract lengths following CldU or IdU pulse labeling. (E) Calculation of replication fork velocity in the indicated genotypes (as described in 'Materials & Methods' section). Examples of representative forks from W & NE cultures are shown for comparison. (F) Assessment of replication fork restart following 1 mM HU treatment using the indicated scheme. Relative tract ratio is calculated by dividing the length of the IdU tract by that of the CldU tract (= 2 under unperturbed conditions). Thus, higher values indicate faster restart following HU removal. Image collected & cropped by CiteAb from the following publication (<https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/gkv691>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Nbs1 Antibody [NB100-143] - UFL1 protein accumulates at DSBs through the MRN complex. a U2OS cells stably expressing UFL1 Tet-on shRNA were treated with doxycycline (Dox) for 3–5 days, & then treated with or without 2 Gy IR. After 30 min, cells were harvested & lysed with NETN buffer. Cell lysates were incubated with UFL1 antibody + Benzodase. The immunoprecipitates were blotted with indicated antibodies. b Immunofluorescence of UFL1 &  $\gamma$ H2AX in U2OS cells irradiated with IR (0.5 Gy). c Triamcinolone acetonide (TA) treatment induces the translocation of RFP-I-SceI-GR fusion protein from the cytoplasm to the nucleus & generates one double strand break at the cutting site. The protein localization was detected by indicated antibodies. d UFL1 foci formation at different time points following 0.5 Gy IR treatment. e, f UFL1 foci formation was analyzed in Mre11 knockdown cells or NBS1 deficient cells (NBST). g NBST cells were transfected with Vector (V) or Flag-NBS1 & treated with or without 2 Gy IR. After 30 min, the cells were lysed & immunoprecipitation with UFL1 antibody with Benzodase treatment was performed. The immunoprecipitates were blotted with indicated antibodies. h The schematic diagram of NBS1 protein domain. i U2OS cells were treated with or without 2 Gy IR, & the cell lysates were pulled down with GST, GST-NBS1 FHA+BRCT (1+2) proteins. After washes, the beads were boiled & analyzed with indicated antibodies. Scale bars, 10  $\mu$ m. Source data are provided as a Source Data file Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30886146>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Moriel-Carretero M, Ovejero S, Gerus-Durand M et al. Fanconi anemia FANCD2 and FANCI proteins regulate the nuclear dynamics of splicing factors. *J. Cell Biol.* 2017-10-13 [PMID: 29030393]

Michelin F, Pitchiaya S, Vitelli V et al. Damage-induced lncRNAs control the DNA damage response through interaction with DDRNAs at individual double-strand breaks *Nat. Cell Biol.* 2017-12-01 [PMID: 29180822]

Jake R. Conway, Riaz Gillani, Jett Crowdis, Brendan Reardon, Jihye Park, Seunghun Han, Breanna Titchen, Mouadh Benamar, Rizwan Haq, Eliezer M. Van Allen Somatic structural variants drive distinct modes of oncogenesis in melanoma *The Journal of Clinical Investigation* 2024-05-14 [PMID: 38758740]

Matthias Altmeyer, Kai J. Neelsen, Federico Teloni, Irina Pozdnyakova, Stefania Pellegrino, Merete Grøfte, Maj-Britt Druedahl Rask, Werner Streicher, Stephanie Jungmichel, Michael Lund Nielsen, Jiri Lukas Liquid demixing of intrinsically disordered proteins is seeded by poly(ADP-ribose) *Nature Communications* 2015-08-19 [PMID: 26286827]

Park D, Gharghabi M, Reczek CR et al. Wwox Binding to the Murine Brca1-BRCT Domain Regulates Timing of Brip1 and CtIP Phospho-Protein Interactions with This Domain at DNA Double-Strand Breaks, and Repair Pathway Choice *International Journal of Molecular Sciences* 2022-03-28 [PMID: 35409089] (In vivo assay)

Wong HT, Luperchio AM, Riley S, Salamango DJ. Inhibition of ATM-directed antiviral responses by HIV-1 Vif PLoS Pathog 2023-09-05 [PMID: 37669285] (Immunocytochemistry/ Immunofluorescence)

Scott WA, Dhanji EZ, Dyakov BJA et al. ATRX proximal protein associations boast roles beyond histone deposition *PLOS Genetics* 2021-11-15 [PMID: 34780483] (Block/Neutralize)

Boichuk S, Bikinieva F, Valeeva E et al. Establishment and Characterization of Multi-Drug Resistant p53-Negative Osteosarcoma SaOS-2 Subline *Diagnostics (Basel)* 2023-08-11 [PMID: 37627905] (Western Blot)

Herok M, Wawrzynow B, Maluszek MJ et al. Chemotherapy of HER2- and MDM2-Enriched Breast Cancer Subtypes Induces Homologous Recombination DNA Repair and Chemoresistance *Cancers (Basel)* 2021-09-07 [PMID: 34572735] (Immunoprecipitation, Western Blot)

Park S, Kwon M, Ju E et al. Targeting phosphomevalonate kinase enhances radiosensitivity via ubiquitination of the replication protein A1 in lung cancer cells *Cancer Science* 2023-07-05 [PMID: 37650703] (ICC/IF, Human)

Nechay M, Wang D, Kleiner RE Inhibition of nucleolar transcription by oxaliplatin involves ATM/ATR kinase signaling *Cell chemical biology* 2023-06-28 [PMID: 37433295]

Schuhwerk H, Kleemann J, Gupta P et al. The EMT transcription factor ZEB1 governs a fitness-promoting but vulnerable DNA replication stress response *Cell reports* 2022-12-13 [PMID: 36516781] (Immunocytochemistry/ Immunofluorescence, Human)

More publications at <http://www.novusbio.com/NB100-143>



## Procedures

### Western Blot protocol for Nbs1 Antibody (NB100-143)

#### Western Blot Procedure

1. Run ~50ug of protein on a 4-20% Tris-glycine mini-gel at 125V for 90 minutes.
  2. Equilibrate gel, nitrocellulose membrane, Whatman paper, and blotting pads in transfer buffer for 15 minutes.
  3. Transfer protein to the membrane at 25V for 90 minutes.
  4. Allow membrane to air-dry.
  5. Block membrane with 1XPBS/3% BSA for 1 hour at room temperature (~23-27 degrees C).
  6. Wash membrane twice, for 5 minutes each, with 1XPBS/0.05% Tween-20 (PBST).
  7. Incubate membrane with NB100-143 (anti-hp95/nibrin), diluted in 1XPBS/1% BSA, for 1 hour at room temperature.
  8. Wash membrane once for 15 minutes, then four times for 5 minutes each, with PBST.
  9. Incubate membrane with goat anti-rabbit IgG-HRP, diluted in 1XPBS/1% BSA, for 1 hour at room temperature.
  10. Wash membrane once for 15 minutes, then four times for 5 minutes each, with PBST.
  11. Detect cross-reacting proteins using Renaissance Chemiluminescence Reagent Plus kit from NEN Life Sciences.
- Note: HeLa whole cell extracts (NB800-PC1) were used as a positive control for this antibody.





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Fax: (44) (0) 1235 533420  
info.EMEA@bio-techne.com

### **General Contact Information**

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Orders: nb-customerservice@bio-techne.com  
General: novus@novusbio.com

### **Products Related to NB100-143**

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NBL1-13497	Nbs1 Overexpression Lysate
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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### **Limitations**

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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