Product Datasheet

53BP1 Antibody - BSA Free
NB100-304

Unit Size: 0.1 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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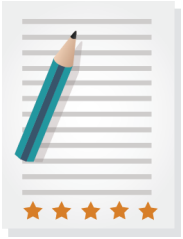
Updated 8/7/2024 v.20.1



NB100-304

53BP1 Antibody - BSA Free

Product Information	
Unit Size	0.1 mg
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	214 kDa

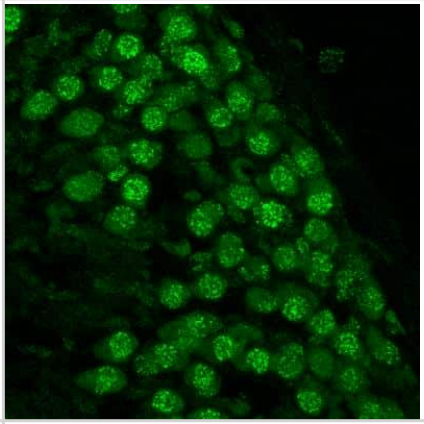
Product Description	
Description	Please note for Lot A5, the dilution recommendations have changed from our previous lot recommendations. You may be required to use the antibody at a higher dilution than previous lots to obtain similar results. Please contact us if you have any questions.
Host	Rabbit
Gene ID	7158
Gene Symbol	TP53BP1
Species	Human, Mouse, Rat, Bat, Bovine, Canine, Fish, Goat, Primate
Reactivity Notes	Human reactivity reported in scientific literature (PMID:32877678). Human and mouse reactivity cited in numerous publications. Primate reactivity reported in scientific literature (PMID: 18389475). Fish reactivity reported in scientific literature (PMID: 25516420). Bat, canine, and bovine reactivity reported in scientific literature (PMID: 27573809). Predicted cross-reactivity based on sequence identity: Chimpanzee (96%), Gorilla (96%), Orangutan (96%), Gibbon (94%), Marmoset (92%), Feline (90%), Porcine (90%), Rabbit (90%), Sheep (90%).
Marker	DNA Double Strand Break Marker
Immunogen	Partial synthetic peptide made to an internal portion of human 53BP1 (between amino acids 350-400) [NP_005648.1].
Notes	Please note for Lot A5, the dilution recommendations have changed from our previous lot recommendations. You may be required to use the antibody at a higher dilution than previous lots to obtain similar results. Please contact us if you have any questions.

Product Application Details	
Applications	Western Blot, Chromatin Immunoprecipitation, Flow Cytometry, Flow (Intracellular), Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, In-situ Hybridization, Chromatin Immunoprecipitation (ChIP), Knockdown Validated, Knockout Validated

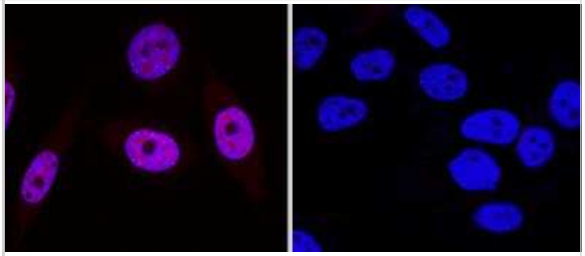
Recommended Dilutions	Western Blot 1 - 2 ug/ml, Chromatin Immunoprecipitation reported in scientific literature (PMID 24591601), Flow Cytometry 1.5 ug/ml, Immunohistochemistry reported in scientific literature (PMID 24987917), Immunocytochemistry/ Immunofluorescence 1:1000-1:5000, Immunoprecipitation reported in scientific literature (PMID 25645366), Immunohistochemistry-Paraffin 1:1000-1:5000. Use reported in scientific literature (PMID 27653664), Immunohistochemistry-Frozen reported by customer review, Immunoblotting reported in multiple pieces of scientific literature, In-situ Hybridization reported in scientific literature (PMID 34988401; 33122290), Flow (Intracellular) 1.5 ug/ml, Chromatin Immunoprecipitation (ChIP) reported in scientific literature (PMID 24591601), Knockout Validated reported in scientific literature (PMID 26601238), Knockdown Validated

Images

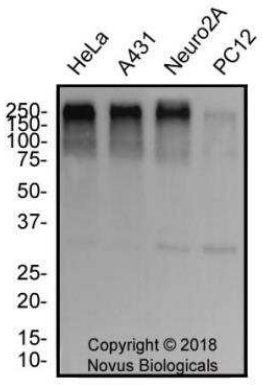
Irradiated cochlear spiral ganglion cells, mouse, frozen section of fixed material. Image from verified customer review.

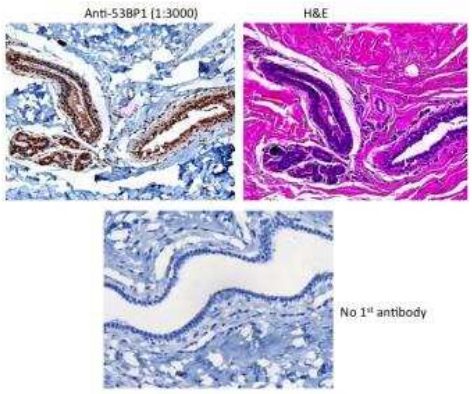
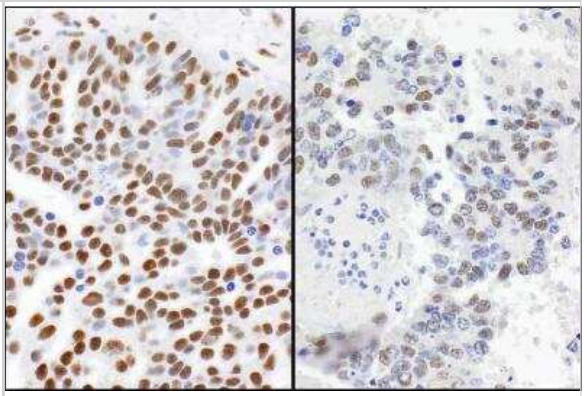
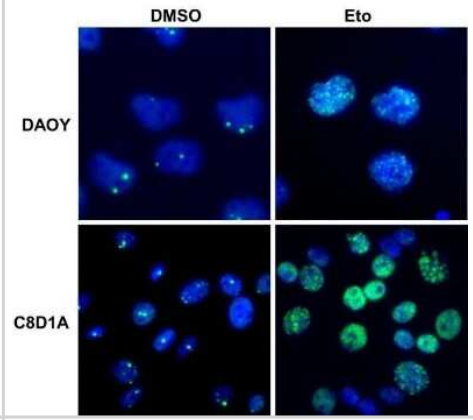
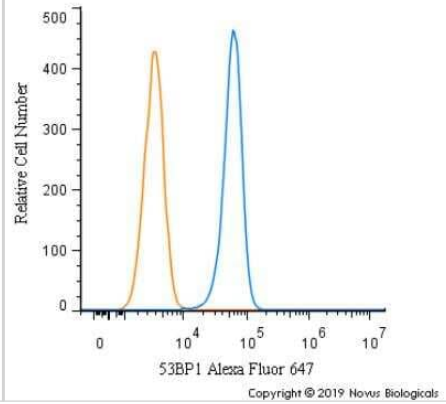


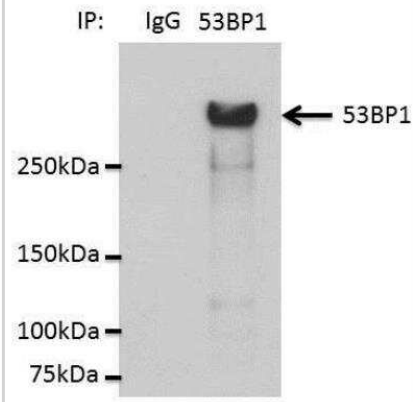
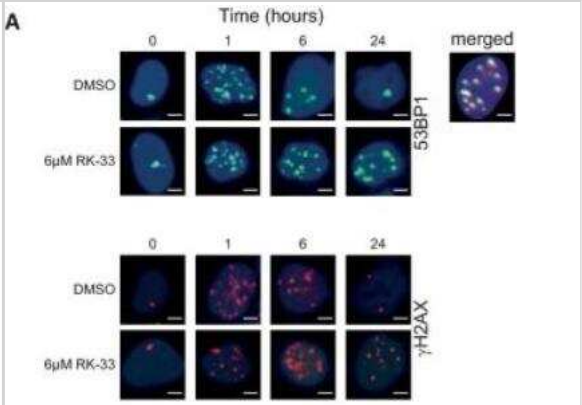
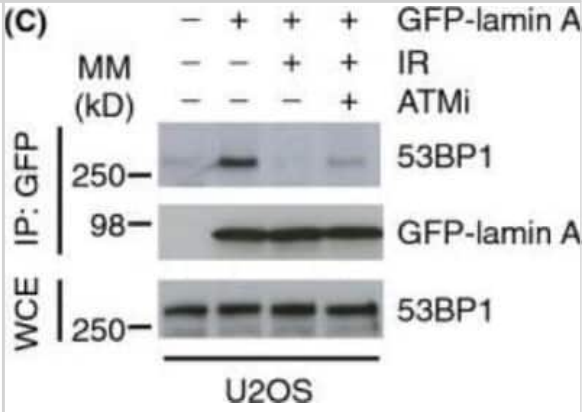
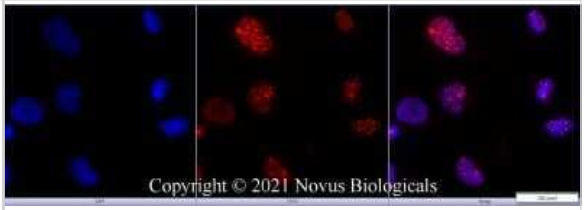
53BP1 was detected in immersion fixed HeLa cells (left) but was not detected in 53BP1 knockout Hela cells (right) using Rabbit Anti-human 53BP1 polyclonal antibody (Catalog #NB100-304) at 0.3 ug/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to nuclei.

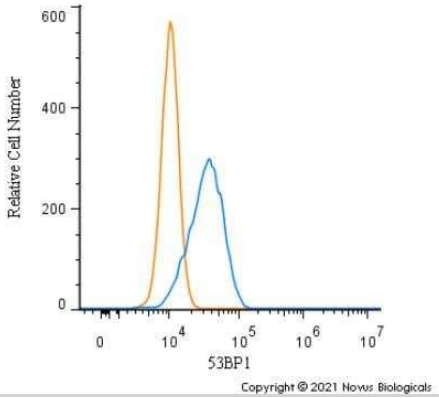
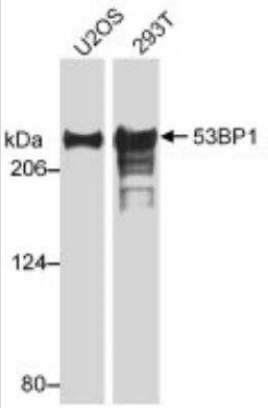
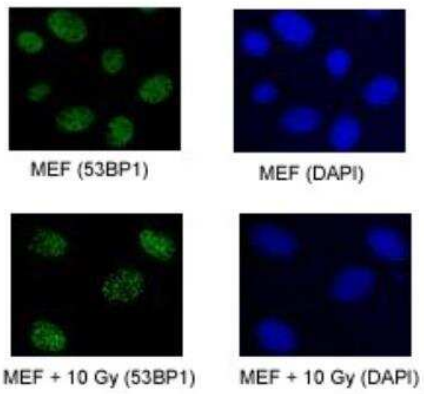
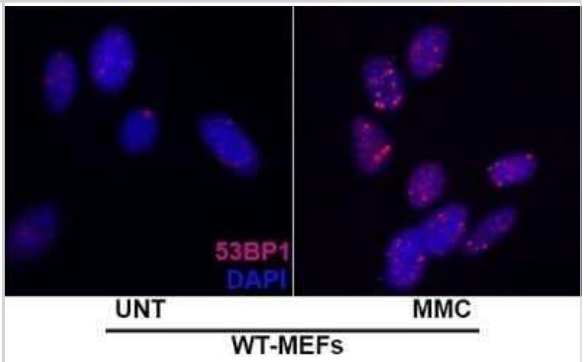


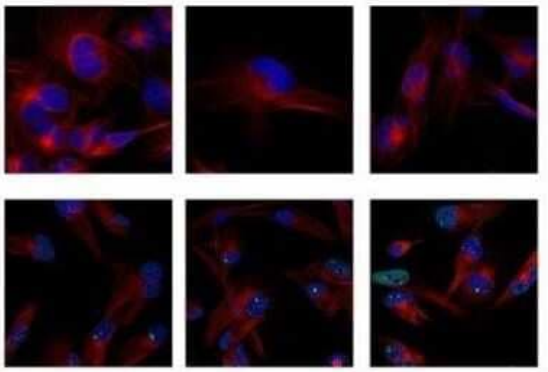
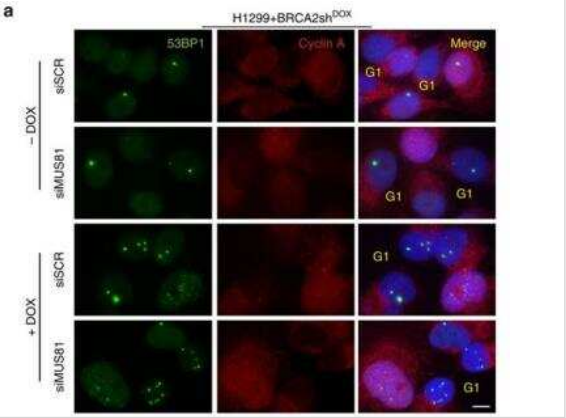
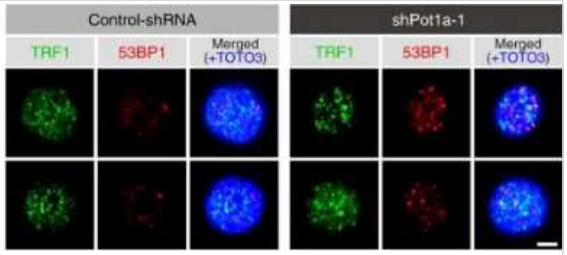
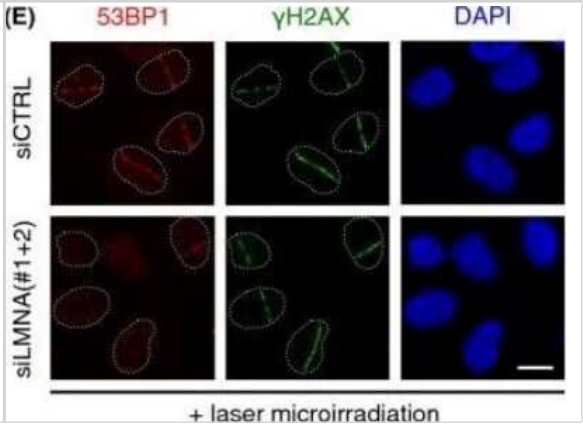
Total protein from HeLa, A431, Neuro2A, and PC12 was separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 1.0 ug/mL in 5% block buffer and detected with an anti-rabbit HRP secondary antibody using chemiluminescence. The observed molecular weight for these samples are ~250 kDa and the theoretical molecular weight is 214 kDa.



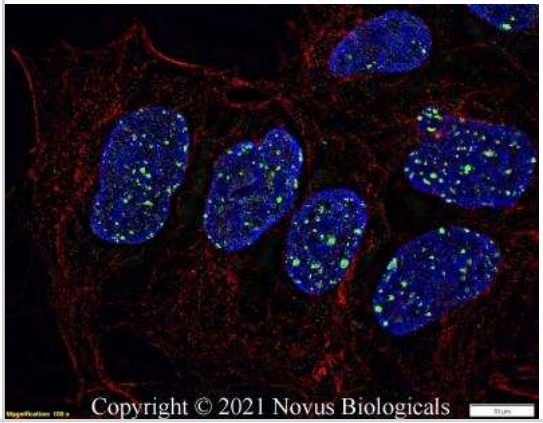
Human breast tumors stained with 53BP1 antibody. Image from verified customer review.	
Detection of Human and Mouse 53BP1 by IHC. Sample: FFPE sections of human ovarian carcinoma (left) and mouse teratoma (right). Antibody: 53BP1 Antibody (Catalog #NB100-304) used at a dilution of 1:1000 (1ug/mL). Detection: DAB.	
Human medulloblastoma (DAOY) and mouse astrocyte (C8D1A) cell lines were exposed for 48 hours to DMSO or 1ug/mL of the DNA damaging agent EPE. Cells were immunostained for 53BP1 (green). The nuclei were counterstained with DAPI (blue). Image from verified customer review.	
An intracellular stain was performed on RH-30 cells with 53BP1 Antibody (Catalog #NB100-304AF647) (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.	

Immunoprecipitation analysis lysates from HCC44 cells in 1% NP40. Image from verified customer review.	
Effect of RK-33 on radiation-induced DNA damage. A Immunofluorescence images showing 53BP1 and gamma-H2AX foci in A549 cells after 2-Gy radiation and A549 cells pre-treated with 6 uM RK-33, 12 h before radiation. Overlap of 53BP1 and gamma-H2AX is seen in the merged picture of the co-immunofluorescence staining. Scale bar is 2 um. Image collected and cropped by CiteAb from the following publication (https://embomolmed.embopress.org/cgi/doi/10.15252/emmm.201404368) licensed under a CC-BY license.	
Lamin A/C-53BP1 interaction is regulated in a DNA damage-dependent manner. U2OS/GFP-lamin A cells were pretreated with 10 um ATMi f for 1 h before exposure to IR (10 Gy, 1 h recovery). Cell extracts were then subjected to immunoprecipitation using GFP-Trap beads, and bound complexes were then analyzed by immunoblotting using 53BP1 and GFP antibodies. WCE represents 1% input. Image collected and cropped by CiteAb from the following publication (https://doi.wiley.com/10.1111/acel.12258), licensed under a CC-BY license.	
HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with 53BP1 Antibody conjugated to DyLight 550 (NB100-304R) at 5 ug/ml for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.	

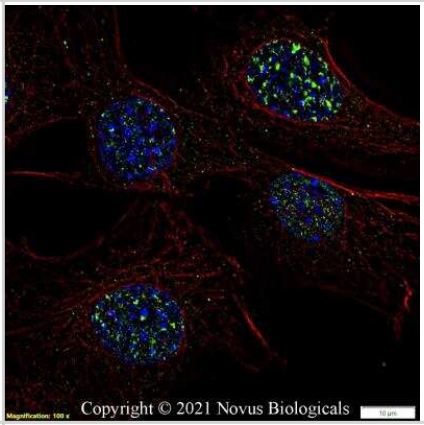
An intracellular stain was performed on Ntera2 cells with 53BP1 Antibody NB100-304 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).	
Whole cell lysate from U2OS or 293T cells. Bands indicate an observed molecular weight of ~220 kDa and the theoretical molecular weight is 214 kDa.	
Upper Panel: 53BP1 foci in proliferating MEFs. Lower Panel: 53BP1 foci in proliferating MEFs exposed to 10 Gy of IR.	
Embryonic Fibroblast cells pre-extraction for 5 mins with CSK buffer. Fixed with 4% PFA and 75% Ethanol. Primary Antibody at 1:1000. Secondary Antibody at 1:1000. Image from verified customer review.	

Upper Panel: Control untreated cells. Lower Panel: Cells exposed to irradiation (10 Gy) and probed for 53BP1 foci. Cells were grown on coverslips, fixed with 4% paraformaldehyde, methanol permeabilized, blocked for 1 h, RT. Incubated with primary antibody (1:200) overnight, washed 3x with PBS, probed with tubulin (Alexa Fluor 594) antibody for 2 h, RT. Washed 3x with PBS, mounted on slides using prolong gold, imaged using Nikon confocal microscope (100x oil). Image from verified customer review.	
MUS81 inhibition in BRCA2-deficient cells causes accumulation of 53BP1 nuclear bodies and G1 arrest. H1299 cells carrying a DOX-inducible BRCA2 shRNA were transfected with control or MUS81 siRNAs. Representative images of cells processed 72 h later for immunostaining with 53BP1 Antibody (green) and anti-cyclin A (red) antibodies. DNA was counterstained with DAPI. Scale bar, 10 um. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/doifinder/10.1038/ncomms15983), licensed under a CC-BY license.	
Pot1a prevents DDR in HSCs. Telomeric DDR in 8 week-old LSK cells upon Pot1a knockdown. Immunocytochemical staining of TRF1 (green). Foci co-stained with TRF1 and 53BP1 were identified as TIFs. Nuclei were stained with TOTO3 (blue). Scale bar, 2 um. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/s41467-017-00935-4), licensed under a CC-BY license.	
Lamin A/C-53BP1 interaction is regulated in a DNA damage-dependent manner. U2OS cells were transfected with siCTRL or siLMNA and subjected to laser micro-irradiation, fixed 15 min later and then processed for immunofluorescence with gamma-H2AX and 53BP1 antibodies. Scale bar, 10 um. Image collected and cropped by CiteAb from the following publication (https://doi.wiley.com/10.1111/acel.12258), licensed under a CC-BY license.	

Ntera2 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-53BP1 Antibody NB100-304 at 2 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse Dylight 550 (Red) at a 1:1000 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.

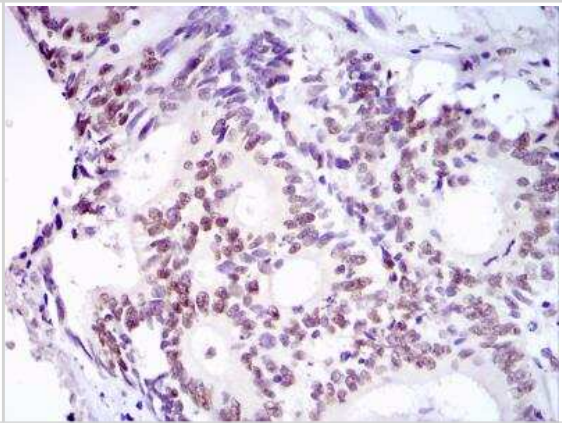


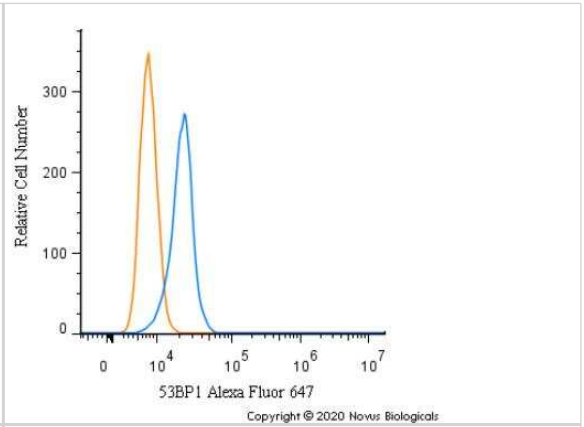
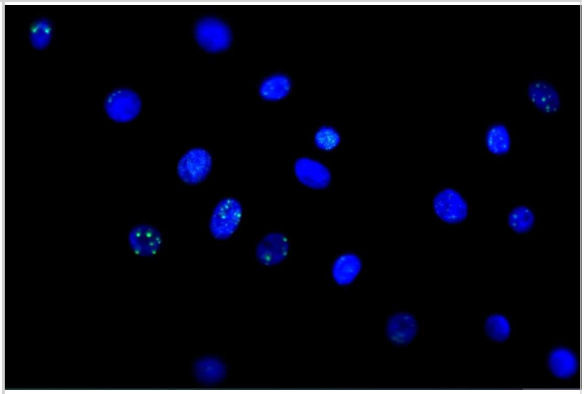
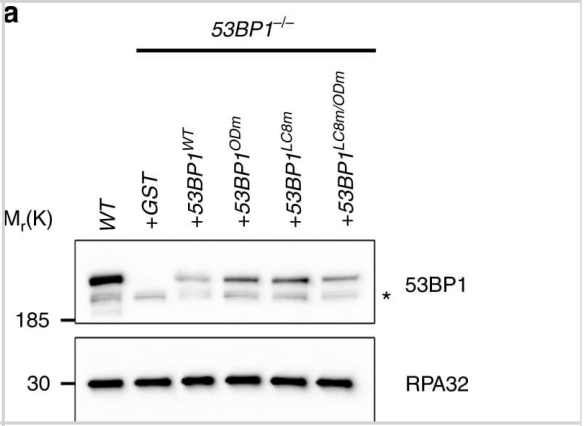
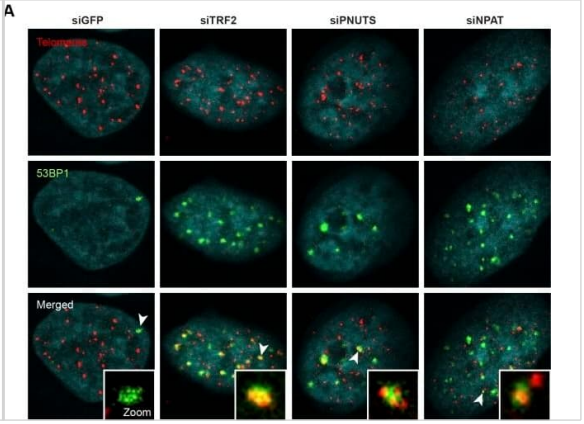
NIH3T3 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-53BP1 Antibody NB100-304 at 2 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse Dylight 550 (Red) at a 1:1000 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



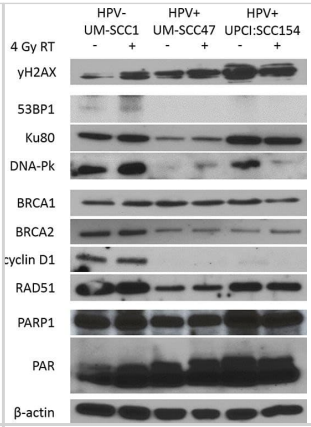
HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with 53PB1 Antibody conjugated to DyLight 550 (NB100-304R) at 5 ug/ml for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.

Staining of 53BP1 in human colon cancer using DAB with hematoxylin counterstain.

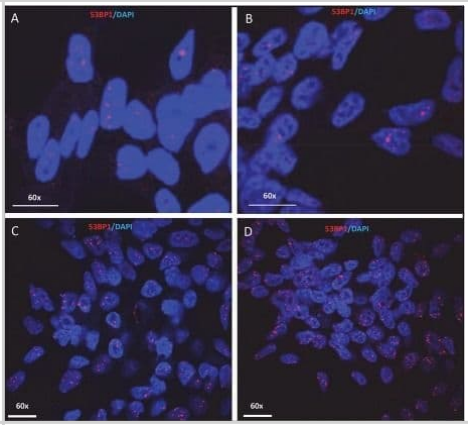


An intracellular stain was performed on A431 cells with 53BP1 Antibody (Catalog #NB100-304AF647) (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.	
For 53BP1 staining, samples were incubated for 1 hour with 1:200 53BP1 primary antibody in PBS/BSA 1%, at room temperature. Green: 53BP Blue: DAPI. Image from verified customer review.	
DYNLL1 is required for 53BP1-dependent p53 responses to Nutlin-3. a Immunoblot analysis of the MCF-7 cell lines used in (a) with anti-53BP1 or anti-RPA32 antibodies prior to N3 treatment. b Quantification of n = 3 independent experiments represented in (a), each performed in triplicate. Mean ± SD. c Indicated parental or stably complemented 53BP1-/- MCF-7 cell lines were incubated in the presence (11 days) or absence (7 days) of Nutlin-3 (4 μM)	
Depletion of PNUTS and NPAT induces a global DDR.(A) Induction of telomere dysfunction–induced foci (TIF) upon candidate knockdown in HeLa cells from Fig 5. Depletion of TRF2 was used as a positive control. 53BP1 was detected by indirect IF and telomeres by FISH with a Cy3-[CCCTAA]3 probe. (B) Frequency distribution of the number of TIFs per cell from (A). n indicates the number of cells analyzed for each condition and red lines mark the mean.	

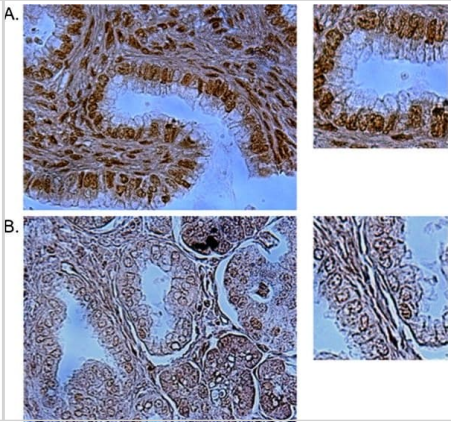
HPV+ HNSCCs have decreased expression of NHEJ and HR proteins including DNA-Pk and BRCA2Cells were treated with mock or 4 Gy radiation, harvested and lysed at 15 minutes post-treatment, and analyzed by western blot for relative expression of indicated proteins. β-actin was used as a loading control. Shown is a representative blot from 2 independent experiments.



LNCaP cells in culture were treated for 24 hrs with DMSO or ENZ, then irradiated with 0 or 1 Gy.Cell were fixed and immunostained for 53BP1 0.5, 1.5 and 24 hrs after irradiation. Immunofluorescent images were obtained by confocal microscopy. White bar = 20 microns. A) DMSO alone, B) ENZ alone, C) 1 Gy, D) ENZ + 1 Gy. White bar = 20 microns.



Representative immunohistochemistry staining for 53BP1 expression in pancreatic adenocarcinoma. (A) is high intensity of 53BP1 expression and (B) is low intensity of 53BP1 expression.



Publications

Grzegorz Sarek, Panagiotis Kotsantis, Phil Ruis, David Van Ly, Pol Margalef, Valerie Borel, Xiao-Feng Zheng, Helen R. Flynn, Ambrosius P. Snijders, Dipanjan Chowdhury, Anthony J. Cesare, Simon J. Boulton CDK phosphorylation of TRF2 controls t-loop dynamics during the cell cycle Nature 2019-10-01 [PMID: 31723267]

Leizhen Wei, Satoshi Nakajima, Stefanie Böhm, Kara A. Bernstein, Zhiyuan Shen, Michael Tsang, Arthur S. Levine, Li Lan DNA damage during the G0/G1 phase triggers RNA-templated, Cockayne syndrome B-dependent homologous recombination Proceedings of the National Academy of Sciences of the United States of America 2015-07-07 [PMID: 26100862]

Craig Davison, Roisin Morelli, Catherine Knowlson, Melanie McKechnie, Robbie Carson, Xanthi Stachtea, Kylie A. McLaughlin, Vivien E. Prise, Kienan Savage, Richard H. Wilson, Karl A. Mulligan, Peter M. Wilson, Robert D. Ladner, Melissa J. LaBonte Targeting nucleotide metabolism enhances the efficacy of anthracyclines and anti-metabolites in triple-negative breast cancer NPJ Breast Cancer 2021-04-06 [PMID: 33824328]

O'Dea R, Santocanale C Non-canonical regulation of homologous recombination DNA repair by the USP9X deubiquitinase J. Cell. Sci. 2020-01-21 [PMID: 31964704]

Michalis Petropoulos, Angeliki Karamichali, Giacomo G. Rossetti, Alena Freudenmann, Luca G. Iacovino, Vasilis S. Dionellis, Sotirios K. Sotiriou, Thanos D. Halazonetis Transcription–replication conflicts underlie sensitivity to PARP inhibitors Nature 2024-03-20 [PMID: 38509368]

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More publications at http://www.novusbio.com/NB100-304

Procedures

Immunohistochemistry-Paraffin protocol for 53BP1 Antibody (NB100-304)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

Western Blot Protocol for 53BP1 Antibody (NB100-304)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

Immunocytochemistry/Immunofluorescence Protocol for 53BP1 Antibody (NB100-304)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

Flow (Intracellular) Protocol for 53BP1 Antibody (NB100-304)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.

a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.

4. Re-suspend cells to a concentration of 1 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabilization buffer to every 1 x 106 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.

a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.

b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).

3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

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NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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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