Product Datasheet

53BP1 Antibody
NB100-305SS

Unit Size: 0.025 ml

Store at 4C. Do not freeze.

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NB100-305SS

53BP1 Antibody

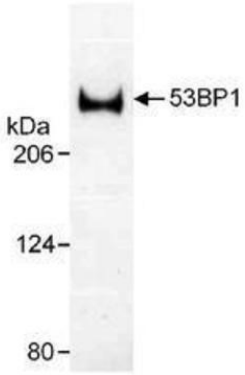
Product Information	
Unit Size	0.025 ml
Concentration	1 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.09% Sodium Azide
Purity	Immunogen affinity purified
Buffer	Tris-citrate/phosphate, pH 7-8
Target Molecular Weight	213 kDa

Product Description	
Host	Rabbit
Gene ID	7158
Gene Symbol	TP53BP1
Species	Human, Mouse, Rat
Species Reactivity	Human has been tested in both WB and ICC/IF, mouse has only been tested in ICC/IF. Rat reactivity reported in scientific literature (PMID: 24244353). Feedback on bovine has been negative.
Marker	DNA Double Strand Break Marker
Immunogen	The epitope recognized by this antibody maps to a region between residues 1925 and the C-terminus (residue 1972) of human 53BP1 (NP_005648.1).
Notes	This anitbody can be used as the primary antibody in a PLA assay with the following as secondary antibodies: NB100-1803, NB100-322, NB100-224, NB100-464, NB100-1707, NB100-2349, NB100-97827, NB500-160, NB200-171

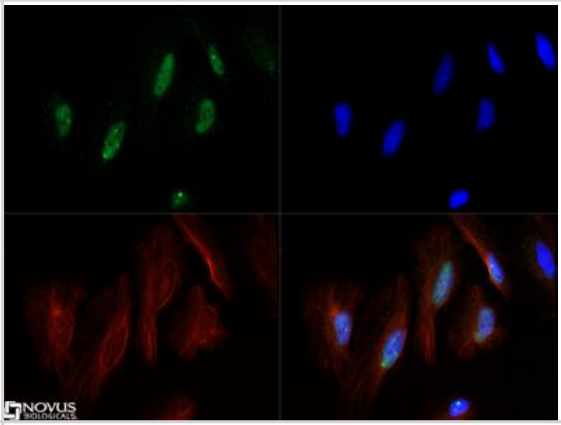
Product Application Details	
Applications	Western Blot, Chromatin Immunoprecipitation, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Proximity Ligation Assay
Recommended Dilutions	Chromatin Immunoprecipitation, Immunocytochemistry/Immunofluorescence 1:50-1:1000, Immunohistochemistry 1:10-1:500, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin 1:10-1:500, Proximity Ligation Assay 1:1000, Western Blot 1:2000-1:10000
Application Notes	This 53BP1 antibody is useful for Immunocytochemistry/Immunofluorescence, Immunohistochemistry on paraffin-embedded sections and Western Blot. Chromatin Immunoprecipitation and Immunohistochemistry-Frozen were reported in scientific literature.

Images

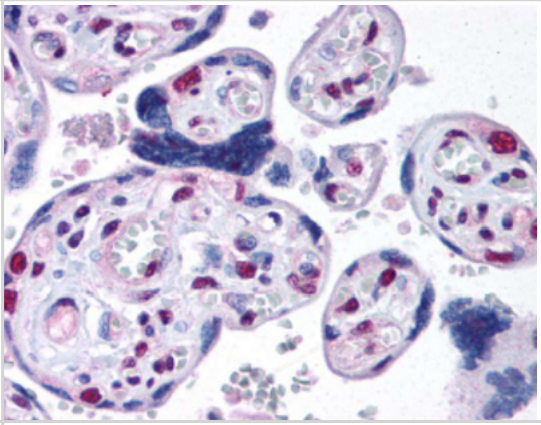
Western Blot: 53BP1 Antibody [NB100-305] - Western Blot analysis of human 53BP1, using NB100-305. Sample: Whole cell lysate (20 ug/lane) from U2Os cells resolved on a 3 to 8 percent trisacetate gel.



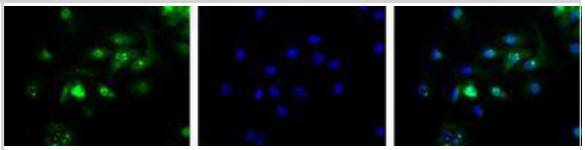
Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-305] - 53BP1 antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



Immunohistochemistry: 53BP1 Antibody [NB100-305] - Placenta, Villi 40X



Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-305] - Detection of 53BP1 (Green) in Hela cells using NB100-305 at a 1:50 dilution. Nuclei (Blue) are counterstained using Hoechst 33258.



Publications

Ibbich N. Immunocytochemical detection of residual double-strand breaks to determine the cellular radiosensitivity under Consideration of chromatin in human fibroblasts / Immuncytochemischer Nachweis residueller Doppelstrangbruchezur Bestimmung der zellularen Strahlenempfindlichkeit unterBerucksichtigung der Chromatinstruktur bei humanen Fibroblasten. Doctor of Medicine Thesis, Medical Faculty of the University of Hamburg, 16 December 2014 Thesis. 2014 (ICC/IF, Human)

Ibbich N. Immuncytochemischer Nachweis residueller Doppelstrangbruche zur Bestimmung der zellularen Strahlenempfindlichkeit unter Berucksichtigung der Chromatinstruktur bei humanen Fibroblasten. Thesis. 2014 (ICC/IF, Human)

Chen L, Zhu X, Zou Y et al. The topoisomerase II catalytic inhibitor ICRF-193 preferentially targets telomeres that are capped by TRF2. Am. J. Physiol., Cell Physiol. 2014 Dec 17 [PMID: 25518961] (ICC/IF, Human)

Dulev S, Tkach J, Lin S, Batada, NN. SET8 methyltransferase activity during the DNA double-strand break response is required for recruitment of 53BP1. EMBO Rep. 2014 Sep 24 [PMID: 25252681]

Klement K, Goodarzi AA. DNA double strand break responses and chromatin alterations within the aging cell. Exp Cell Res. 2014 Sep 08 [PMID: 25218945]

Lu H, Fang EF, Sykora P et al. Senescence induced by RECQL4 dysfunction contributes to Rothmund-Thomson syndrome features in mice. Cell Death Dis. 2014 May 16 [PMID: 24832598] (ICC/IF, Human)

Clynes D, Jelinska C, Xella B et al. ATRX Dysfunction Induces Replication Defects in Primary Mouse Cells. PLoS ONE 3/21/2014 [PMID: 24651726] (ICC/IF, Mouse)

Kato K, Nakajima K, Ui A et al. Fine-Tuning of DNA Damage-Dependent Ubiquitination by OTUB2 Supports the DNA Repair Pathway Choice. Mol. Cell 2014 Feb 24 [PMID: 24560272] (ICC/IF, Human)

Dey M, Patra S, Su LY, Segall AM. Tumor Cell Death Mediated by Peptides That Recognize Branched Intermediates of DNA Replication and Repair. PLoS One. 2013 Nov 14 [PMID: 24244353] (ICC/IF, Rat)

Zimmermann M, Lottersberger F, Buonomo SB et al. 53BP1 regulates DSB repair using Rif1 to control 5' end resection. Science. 2013 Feb 8 [PMID: 23306437] (WB, Mouse)

Chapman JR, Sossick AJ, Boulton SJ et al. BRCA1-associated exclusion of 53BP1 from DNA damage sites underlies temporal control of DNA repair. J Cell Sci. 2012 Aug 1 [PMID: 22553214] (ICC/IF, Human)

Nakada S, Tai I, Panier S et al. Non-canonical inhibition of DNA damage-dependent ubiquitination by OTUB1. Nature. 2010 Aug 19 [PMID: 20725033] (ICC/IF, Human)

More publications at http://www.novusbio.com/NB100-305

Procedures

Immunohistochemistry Protocol for 53BP1 Antibody (NB100-305)
IHC-FFPE sections

I. Deparaffinization:

A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes

II. Quench Endogenous Peroxidase:

A. Place slides in peroxidase quenching solution: 15-30 minutes.

To Prepare 200 ml of Quenching Solution: Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol. Use within 4 hours of preparation style

B. Place slides in distilled water: 2 changes for 2 minutes each.

III. Retrieve Epitopes:

A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96 degrees Celcius.
B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
D. Slowly add distilled water to further cool for 5 minutes.
E. Rinse slides with distilled water. 2 changes for 2 minutes each

IV. Immunostaining Procedure:

A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).
B. Flood slide with Wash Solution. Do not allow tissue sections to dry for the rest of the procedure.
C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.
E. Wash slides with Wash Solution: 3 changes for 5 minutes each
F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes. Check development with microscope.
I. Wash slides with Wash Solution: 3 changes for 5 minutes each.
J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
M. Rinse slides in distilled water.
N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip. Lay slides on a flat surface to dry prior to viewing under microscope.

NOTES:

-Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.
-Prior to deparaffinization, heat slides overnight in a 60 degrees Celcius oven.
-All steps in which Xylene is used should be performed in a fume hood.
-For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.
-For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.
-200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200 ul may be used.
-5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.
-Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1.5 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).

Immunocytochemistry/Immunofluorescence Protocol for 53BP1 Antibody (NB100-305)
Immunocytochemistry Protocol

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

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

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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.

For more information on our guarantee, please visit www.novusbio.com/guarantee.