Product Datasheet

53BP1 Antibody
NB100-304

Unit Size: 0.1 ml

Store at -20 °C.

Reviews: 11  Publications: 386

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB100-304

Updated 7/28/2019 v.20.1

Earn rewards for product reviews and publications.
Submit a publication at www.novusbio.com/publications
Submit a review at www.novusbio.com/reviews/destination/NB100-304
## NB100-304
### 53BP1 Antibody

<table>
<thead>
<tr>
<th><strong>Product Information</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unit Size</strong></td>
<td>0.1 ml</td>
</tr>
<tr>
<td><strong>Concentration</strong></td>
<td>1 mg/ml</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>Store at -20 °C.</td>
</tr>
<tr>
<td><strong>Clonality</strong></td>
<td>Polyclonal</td>
</tr>
<tr>
<td><strong>Preservative</strong></td>
<td>0.05% Sodium Azide</td>
</tr>
<tr>
<td><strong>Isotype</strong></td>
<td>IgG</td>
</tr>
<tr>
<td><strong>Purity</strong></td>
<td>Immunogen affinity purified</td>
</tr>
<tr>
<td><strong>Buffer</strong></td>
<td>PBS</td>
</tr>
<tr>
<td><strong>Target Molecular Weight</strong></td>
<td>214 kDa</td>
</tr>
</tbody>
</table>

### Product Description

**Host**
- Rabbit

**Gene ID**
- 7158

**Gene Symbol**
- TP53BP1

**Species**
- Human, Mouse, Rat, Fish, Goat, Primate

**Reactivity Notes**
- Human and mouse reactivity cited in numerous publications. Primate reactivity reported in scientific literature (PMID: 18389475). Fish reactivity reported in scientific literature (PMID: 25516420).

**Marker**
- DNA Double Strand Break Marker

**Immunogen**
The epitope recognized by this antibody maps to a region between residues 350 and 400 of human 53BP1 [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), Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Immunocytochemistry, Knockout Validated

**Recommended Dilutions**
- Western Blot 1:5000 - 1:25000, Chromatin Immunoprecipitation, Flow Cytometry 1.5 ug/mL, Immunohistochemistry, Immunocytochemistry/Immunofluorescence 1:1000 - 1:5000, Immunoprecipitation, Immunohistochemistry-Paraffin 1:1000 - 1:5000, Immunohistochemistry-Frozen, Flow (Intracellular), Immunocytochemistry 1:200, Knockout Validated

**Application Notes**
- Immunohistochemistry-Paraffin (PMID: 27653664) and Immunohistochemistry (PMID: 24987917) were reported in scientific literature. Frozen section data from customer review. Epitope retrieval with citrate buffer pH 6.0 is recommended for FFPE tissue sections. Use in chromatin immunoprecipitation reported in scientific literature (PMID: 24591601). Use in Immunoprecipitation reported in scientific literature (PMID: 25645366). Knockout Validation was reported in scientific literature (PMID: 26601238).
Western Blot: 53BP1 Antibody [NB100-304] - 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 anti-53BP1 in 5% block buffer and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.

Knockout Validated: 53BP1 Antibody [NB100-304] - 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.

Immunohistochemistry-Frozen: 53BP1 Antibody [NB100-304] - Irradiated cochlear spiral ganglion cells, mouse, frozen section of fixed material. Image from verified customer review.

Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-304] - 53BP1 foci in proliferating MEFs; both normal and exposed to 10 Gy of IR.
<table>
<thead>
<tr>
<th><strong>Immunohistochemistry-Paraffin: 53BP1 Antibody [NB100-304]</strong> - <strong>Detection of Human and Mouse 53BP1 by IHC. Sample: FFPE sections of human ovarian carcinoma (left) and mouse teratoma (right). Antibody: NB100-304 used at a dilution of 1:1000 (1μg/mL). Detection: DAB.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flow Cytometry: 53BP1 Antibody [NB100-304] - 1 million Jurkat cells were fixed, permeabilized, and stained with 1.5 μg/mL anti-53BP1 NB100-304 in a 150 μL reaction. Isotype control (black), anti-53BP1 (red).</strong></td>
</tr>
<tr>
<td><strong>Immunohistochemistry-Paraffin: 53BP1 Antibody [NB100-304] - Human breast tumors stained with 53BP1 antibody. Image from verified customer review.</strong></td>
</tr>
<tr>
<td><strong>Western Blot: 53BP1 Antibody [NB100-304] - Whole cell lysate from U2OS or 293T cells.</strong></td>
</tr>
</tbody>
</table>
Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-304] - PC12 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton-X100. The cells were incubated with anti-53BP1 Antibody at 2 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

Flow Cytometry: 53BP1 Antibody [NB100-304] - An intracellular stain was performed on NIH3T3 cells with 53BP1 Antibody NB100-304AF488 (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 488.

Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-304] - 53BP1 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using 1 ug/mL of rabbit anti- 53BP1 polyclonal (NB100-304, Novus Biologicals). Cells were stained donkey anti-goat IgGNL557 and counterstained with DAPI (blue).

Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-304] - A431 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton-X100. The cells were incubated with anti-53BP1 at 2 ug/mL overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. 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 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.
**Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-304]** - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton-X100. The cells were incubated with anti-53BP1 at 2 ug/mL overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. 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 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

**Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-304]** - Neuro2a cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton-X100. The cells were incubated with anti-53BP1 at 2 ug/mL overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. 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 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

**Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-304]** - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton X-100. The cells were incubated with anti-53BP1 conjugated to DyLight 550 [NB100-304R] at 10ug/mL for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

**Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-304]** - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton X-100. The cells were incubated with anti-53BP1 conjugated to Alexa Fluor 488 [NB100-304AF488] at 10 ug/mL for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.
Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-304] - Human medulloblastoma (DAOY) and mouse astrocyte (C8D1A) cell lines were exposed for 48 hours to DMSO or 1ug/mL of the DNA damaging agent Etoposide. Cells were immunostained for 53BP1 (green). The nuclei were counterstained with DAPI (blue). Image from verified customer review.

Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-304] - 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.

Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-304] - 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.

Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-304] - NIH3T3 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton-X100. The cells were incubated with anti-53BP1 Antibody at 2 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

Flow (Intracellular): 53BP1 Antibody [NB100-304] - An intracellular stain was performed on Neuro2A cells with NB100-304F (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 10 µg/mL for 30 minutes at room temperature. Both antibodies were conjugated to FITC.

Flow (Intracellular): 53BP1 Antibody [NB100-304] - An intracellular stain was performed on Neuro2A cells with NB100-304G (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 µg/mL for 30 minutes at room temperature. Both antibodies were conjugated to DyLight 488.

Flow Cytometry: 53BP1 Antibody [NB100-304] - An intracellular stain was performed on HeLa cells with 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 µg/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.
Flow Cytometry: 53BP1 Antibody [NB100-304] - An intracellular stain was performed on HeLa cells with 53BP1 Antibody NB100-304AF488 (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 μg/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.

Immunoprecipitation: 53BP1 Antibody [NB100-304] - Lysates from HCC44 cells in 1% NP40. Image from verified customer review.
<table>
<thead>
<tr>
<th>Publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancevska A, Pfeiffer V, Feretzaki M, Ahmed W SMCHD1 Promotes ATM-dependent DNA Damage Signaling and Repair of Uncapped Telomeres bioRxiv (ICC/IF, Human)</td>
</tr>
<tr>
<td>Ge Y, Wu S, Zhang Z et al. Inhibition of p53 and/or AKT as a new therapeutic approach specifically targeting ALT cancers Protein Cell May 21 2019 12:00AM [PMID: 31115790] (ICC/IF, Human)</td>
</tr>
</tbody>
</table>

More publications at [http://www.novusbio.com/NB100-304](http://www.novusbio.com/NB100-304)
Procedures
Immunocytochemistry Protocol for 53BP1 Antibody (NB100-304)

Materials
1) 1 Phosphate buffered saline (pH 7.6): NaCl 137mmol/L, KCl 2.7mmol/L, Na2HPO4 4.3mmol/L, KH2PO4 1.4 mmol/L
2) Citrate buffer, 0.01 M, pH6.0, Sodium Citrate 3g, Citric acid 0.4g
3) 3% Hydrogen peroxide
4) Primary antibody
5) Blocking serum (normal serum)
6) Biotinylated secondary antibody
7) DAB staining kit

Methods
1. Dewax and hydration of slides using xylene and EtOH:
   Dry slides for 20 min in a 60 C oven
   Add Xylene, 2 x 10 min
   100%, 95%, 80%, and 70% EtOH, 5 min each EtOH concentration
   Rinse in PBS, 5'

2 Antigen retrieval method (only for paraffin slides)
1a. High-pressure antigen retrieval procedure (recommended method)
   Place slides in a glass slide holder (ensure that the slide holder is completely filled with slides, slides without sections
   if necessary, to ensure even heating. The entire slide holder is immersed in 1000 ml of Citrate buffer (0.01M, pH6.0)
   within a pressure cooker
   Once steam is produced, and ONLY when steam is visible, from the pressure cooker (usually 15-20 min), the
   required high-pressure will have been reached, and slides will be incubated for 2 min.
   Turn off heat, and allow buffer and slides to cool to room temperature
   Slides are then rinsed in PBS for 5 minutes
   2. Add 3% hydrogen peroxide solution, 10'at RT, then PBS, 3X5'
   3. Normal blocking serum, 20'at RT
   4. Incubate with Primary Ab, 4C overnight or 1.5 hours at 37C
   5. Rinse with PBS, 3 X 5' each rinse
   6. Add Biotin-conjugated secondary antibody, 10'at RT
   7. Rinse with PBS, 3 X 5' each rinse
   8. Add Streptavidin-Peroxidase, 10'at RT
   9. Rinse with PBS, 3 X 5' each rinse
   10. Staining with DAB solution, 2-5'under microscope
   11. Stop the reaction by washing in tap water
   12. Counterstain in Haematoxylin for 3-5 minutes
   13. 75%, 80%, 95% and 100% ethanol, 5x2', xylene 2 x 10'
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 100% guarantee, please visit www.novusbio.com/guarantee

Earn gift cards/discounts by submitting a review: www.novusbio.com/reviews/submit/NB100-304

Earn gift cards/discounts by submitting a publication using this product: www.novusbio.com/publications