Product Datasheet

53BP1 Antibody
NB100-305

Unit Size: 0.1 ml

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

Reviews: 5  Publications: 104

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### 53BP1 Antibody

#### Product Information

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</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 4°C short term. Aliquot and store at -20°C long term. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td><strong>Clonality</strong></td>
<td>Polyclonal</td>
</tr>
<tr>
<td><strong>Preservative</strong></td>
<td>0.02% 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>
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#### Product Description

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td><strong>Host</strong></td>
<td>Rabbit</td>
</tr>
<tr>
<td><strong>Gene ID</strong></td>
<td>7158</td>
</tr>
<tr>
<td><strong>Gene Symbol</strong></td>
<td>TP53BP1</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td>Human, Mouse, Rat, Bat, Bovine, Canine, Naked mole-rat</td>
</tr>
<tr>
<td><strong>Reactivity Notes</strong></td>
<td>Use in Naked mole-rat reported in scientific literature (PMID:33608273). Human has been tested in both WB and ICC/IF, mouse has only been tested in ICC/IF. Feedback on bovine has been negative. Rat reactivity reported in scientific literature (PMID: 24244353). Bat, canine, and bovine reactivity reported in scientific literature (PMID: 27573809). Predicted cross-reactivity based on sequence identity: Chimpanzee (100%), Equine (100%), Feline (100%), Gibbon (100%), Gorilla (100%), Hamster (100%), Marmoset (100%), Orangutan (100%), Panda (100%), Porcine (100%), Rabbit (100%), Sheep (100%).</td>
</tr>
<tr>
<td><strong>Marker</strong></td>
<td>DNA Double Strand Break Marker</td>
</tr>
<tr>
<td><strong>Immunogen</strong></td>
<td>The epitope recognized by 53BP1 Antibody maps to a region between residues 1925 and the C-terminus (residue 1972) of human 53BP1 (NP_005648.1).</td>
</tr>
<tr>
<td><strong>Notes</strong></td>
<td>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</td>
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</table>

#### Product Application Details

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Applications</strong></td>
<td>Western Blot, Chromatin Immunoprecipitation, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin</td>
</tr>
<tr>
<td><strong>Application Notes</strong></td>
<td>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.</td>
</tr>
</tbody>
</table>
**Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-305]** - Paraformaldehyde (4%) fixed Saos-2 cells (human osteosarcoma cell line) were stained with anti-53BP1 antibody followed by Alexa Fluor 488 secondary antibody. ICC/IF image submitted by a verified customer review.

![Immunocytochemistry/Immunofluorescence](image1)

**Western Blot: 53BP1 Antibody [NB100-305]** - Western blot of whole cell lysate (20 ug/lane) from U2Os cells resolved on a 3 to 8 percent trisacetate gel. A band appears at approximately the theoretical molecular weight of 214 kDa.

![Western Blot](image2)

**Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-305]** - Exosomes modulate the repair of DNA DSBs in irradiated recipient cells. Representative images of 53BP1 foci in BHY cells 6 hours after 2 Gy and transfer of BHY exosomes isolated 24 hours after irradiation with 0, 3, 6 or 9 Gy (53BP1 foci green, nuclei blue). Image collected and cropped by CiteAb from the following publication ([http://dx.plos.org/10.1371/journal.pone.0152213](http://dx.plos.org/10.1371/journal.pone.0152213)), licensed under a CC-BY licence.

![Immunocytochemistry/Immunofluorescence](image3)

**Immunohistochemistry: 53BP1 Antibody [NB100-305]** - Immunohistochemical staining of placental villi with 53BP1 Antibody (Catalog #NB100-305) at 40X magnification.

![Immunohistochemistry](image4)
Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-305] - 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 NB100-305 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.

Flow Cytometry: 53BP1 Antibody [NB100-305] - An intracellular stain was performed on Ntera2 cells with 53BP1 Antibody NB100-305 (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).

Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-305] - 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 NB100-305 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.

Flow Cytometry: 53BP1 Antibody [NB100-305] - An intracellular stain was performed on HeLa cells with 53BP1 Antibody (Catalog #NB100-305AF488) (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.
### Publications

Augereau A, Mariotti M, Pousse M et al. Naked mole rat TRF1 safeguards glycolytic capacity and telomere replication under low oxygen Science advances Feb 1 2021 12:00AM [PMID: 33608273] (ICC/IF, Naked mole-rat)

**Details:**
Naked mole-rat (Heterocephalus glaber)

Jang S, Redon C, Fu H et al. RepID-deficient cancer cells are sensitized to a drug targeting p97/VCP segregase Molecular & Cellular Toxicology Feb 1 2021 12:00AM

Affandi T, Ohm AM, Gaillard D et al. Tyrosine kinase inhibitors protect the salivary gland from radiation damage by increasing DNA double strand break repair The Journal of biological chemistry Feb 8 2021 12:00AM [PMID: 33571522] (Human)

Oizumi T, Ohno R, Yamabe S et al. Repair Kinetics of DNA Double Strand Breaks Induced by Simulated Space Radiation Life (Basel, Switzerland) Dec 10 2020 12:00AM [PMID: 33321941] (ICC/IF, Human)

Lototska L, Yue J X et al. Human RAP1 specifically protects telomeres of senescent cells from DNA damage. EMBO Rep Mar 4 2020 12:00AM [PMID: 32096305] (ICC/IF, Human)


Damen M, Soroka E, Khatif H et al. Epidermal stratification is uncoupled from centrosome-dependent cell division orientation of the basal progenitors bioRxiv Jul 25 2020 12:00AM (ICC/IF, Mouse)

Gerakopoulos V, Ngo P, Tsiokas L Loss of polycystins suppresses deciliation via the activation of the centrosomal integrity pathway Life Sci Alliance Sep 1 2020 12:00AM [PMID: 32651191] (ICC/IF, WB, Mouse)

Malaquin N, Vancayseele A, Gilbert S et al. DNA Damage- But Not Enzalutamide-Induced Senescence in Prostate Cancer Promotes Senolytic Bcl-xL Inhibitor Sensitivity Cells Jul 1 2020 12:00AM [PMID: 32630281]

de Krijger I, van der Torre J, Peuscher MH et al. H3K36 dimethylation by MMSET promotes classical non-homologous end-joining at unprotected telomeres Oncogene May 29 2020 12:00AM [PMID: 32472076] (ISH, ICC/IF, Mouse)


More publications at [http://www.novusbio.com/NB100-305](http://www.novusbio.com/NB100-305)
Immunohistochemistry protocol for 53BP1 Antibody (NB100-305)

53BP1 Antibody: https://www.novusbio.com/products/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 Celsius 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)**

**53BP1 Antibody:** [https://www.novusbio.com/products/53bp1-antibody_nb100-305](https://www.novusbio.com/products/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 1:25,000 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.*
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

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Products Related to NB100-305

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<tr>
<th>Code</th>
<th>Description</th>
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<tbody>
<tr>
<td>NB820-59248</td>
<td>Human Placenta Whole Tissue Lysate (Adult Whole Normal)</td>
</tr>
<tr>
<td>HAF008</td>
<td>Goat anti-Rabbit IgG Secondary Antibody [HRP (Horseradish Peroxidase)]</td>
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<tr>
<td>NB7160</td>
<td>Goat anti- Rabbit, Rat IgG (H+L) Secondary Antibody [HRP]</td>
</tr>
<tr>
<td>NBP2-24891</td>
<td>Rabbit, Mouse IgG Isotype Control</td>
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