Product Datasheet

NB100-1803

Unit Size: 0.1 ml

Store at 4C. Do not freeze.

Publications: 11

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Updated 8/2/2017 v.20.1

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**NB100-1803**


### Product Information

<table>
<thead>
<tr>
<th><strong>Unit Size</strong></th>
<th>0.1 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration</strong></td>
<td>0.2 mg/ml</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>Store at 4°C. Do not freeze.</td>
</tr>
<tr>
<td><strong>Clonality</strong></td>
<td>Polyclonal</td>
</tr>
<tr>
<td><strong>Preservative</strong></td>
<td>0.09% Sodium Azide</td>
</tr>
<tr>
<td><strong>Purity</strong></td>
<td>Immunogen affinity purified</td>
</tr>
<tr>
<td><strong>Buffer</strong></td>
<td>TBS and 0.1% BSA</td>
</tr>
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</table>

### Product Description

<table>
<thead>
<tr>
<th><strong>Host</strong></th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<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</td>
</tr>
<tr>
<td><strong>Marker</strong></td>
<td>DNA Double Strand Break Marker</td>
</tr>
<tr>
<td><strong>Immunogen</strong></td>
<td>Immunogen for this antibody was a phosphorylated synthetic peptide, which represented a portion of human p53 Binding Protein 1 (GeneID 7158) around serine 25 according to the numbering given in entry NP_005648.1.</td>
</tr>
<tr>
<td><strong>Notes</strong></td>
<td>This antbody can be used as the primary antibody in a PLA assay with the following as complementing antibodies: NB100-97831, NB110-40543, NB100-322, NB200-171, NB100-68265</td>
</tr>
</tbody>
</table>

### Product Application Details

<table>
<thead>
<tr>
<th><strong>Applications</strong></th>
<th>Western Blot, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Proximity Ligation Assay</th>
</tr>
</thead>
</table>

### Images

Western Blot: 53BP1 [p Ser25] Antibody [NB100-1803] - Samples: Whole cell lysate (30 ug or 15 ug for WB; 1 mg for IP, 20% of IP loaded) from 293T cells that were mock treated or treated with NCS (200 ng/ml; 1h). Affinity purified rabbit anti-Phospho 53BP1 antibody at 0.04 ug/ml (A) and 0.1 ug/ml (B) for WB. 53BP1 was immunoprecipitated using affinity purified rabbit anti-53BP1 antibody NB100-304. Detection: Chemiluminescence with exposure times of 3 seconds (A and B).

Flow Cytometry: 53BP1 [p Ser25] Antibody [NB100-1803] - Flow cytometric analysis of phospho 53bp1 (S25). Jurkat cells were treated with neocarzinostatin (NCS) for 1.5 hrs. 1 Million cells were fixed, permeabilized, and stained with 1.5 ug/ml antiphospho 53bp1 (S25) NB100-1803 in a 150 ul reaction. Isotype control (black), untreated (red), NCS treated (blue).


Proximity Ligation Assay: 53BP1 [p Ser25] Antibody [NB100-1803] - Secondary-conjugate Duolink In Situ PLA in Hela cells. goat anti-human CHK1 (NB100-311) and rabbit anti-human phospho-53BP1 [S25] (NB100-1803). Image merged in DAPI (2ms), FITC/Green (25ms) and TRITC/Orange (250ms) exposures, 4X magnification. Control images may not have counterstained cytoplasm.

Proximity Ligation Assay: 53BP1 [p Ser25] Antibody [NB100-1803] - Secondary-conjugate Duolink In Situ PLA in Hela cells. goat anti-human RelA (NBP2-22297) and rabbit anti-human phospho-53BP1 [S25] (NB100-1803). Image merged in DAPI (2ms), FITC/Green (25ms) and Orange (250ms) exposures, 4X magnification. Control images may not have counterstained cytoplasm.

Publications


Procedures

Flow Cytometry Protocol for 53BP1 Antibody (NB100-1803)

Flow Cytometry Protocol for Indirect Intracellular Staining of Cultured Cells Grown in Suspension:

Reagents Required:

- 16\% parafomaldehyde (PFA) (Electron Microscopy Sciences RT 15710)
- 90\% Methanol (ice cold)
- PBS (Phosphate Buffered Saline pH 7.2)
- 11.6 g sodium chloride
- 100 ml 1 M phosphate buffer
- 0.26 M 4 M sodium hydroxide
- DI H2O to 1 liter
- ICSB (IntraCellular Staining Buffer)
- 495 ml PBS
- 5 ml FBS (fetal bovine serum)
- 0.36 ml 9\% sodium azide
- 12 x 75 FACS tubes (BD Falcon #352054)

Cell Fixation and Permeabilization:

1. For cells grown in suspension, add fresh 16\% PFA to achieve a final concentration of 1.54\% directly to the cells in media (1-2 x 10^6 cell/ml).
2. Fix cells at room temperature (RT) for 10 minutes.
3. Incubate and fix on ice for an additional 30 minutes.
4. Spin fixed cells at 250 x g for 5 minutes at 20\C.
5. Pour off media + PFA into dedicated PFA waste containier.
6. Permeabilize cells by resuspending in ice cold 90\% methanol to a final cell concentration of 1 X 10^6 cells/ml.
7. Cells may be stored at -70 to -20\C for up to a month.

Intracellular Staining:

1. Aliquot 1 ml (1 x 10^6 cells) of cells in methanol for each tube/sample.
2. Spin at 250 x g for 5 minutes at 20\C, break set to slow (perform all subsequent spins at these conditions)
3. Pour off methanol.
4. Resuspend cells in 1 ml ICSB for wash.
5. Pour off wash and blot tube on paper towels.
6. Resuspend cells in 50 mcl of primary antibody at desired dilution.
7. Incubate at RT for 1 hour.
8. Add 1 ml of ICSB.
9. Spin.
10. Pour off and blot tube.
11. Resuspend in 100 mcl of conjugated secondary antibody at desired dilution.
12. Incubate 30 minutes at RT in dark.
13. Add 1 ml ICSB for 2nd wash.
14. Spin, pour off, and blot tube.
15. Resuspend in 100 mcl ICSB.
16. Analyze on a flow cytometer.

Notes:

This is a recommended protocol, and additional optimization may be required for any individual experiment.
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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