

Product Datasheet

53BP1 [p Ser25] Antibody NB100-1803

Unit Size: 0.1 ml

Store at 4C. Do not freeze.

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NB100-1803

53BP1 [p Ser25] Antibody

Product Information	
Unit Size	0.1 ml
Concentration	0.2 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.09% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	TBS and 0.1% BSA

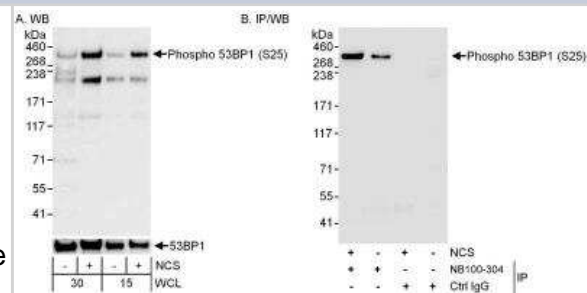
Product Description	
Description	This antibody can be used as the primary antibody in a PLA assay with the following as complementing antibodies: NB100-97831, NB110-40543, NB100-322, NB200-171, NB100-68265
Host	Rabbit
Gene ID	7158
Gene Symbol	TP53BP1
Species	Human, Mouse
Marker	DNA Double Strand Break Marker
Immunogen	53BP1 [p Ser25] Antibody was made to 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.
Notes	This antibody 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

Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Western Blot 1:2000-1:10000, Flow Cytometry 25 ug per 1 million cells in a 150 mcl reaction, Immunohistochemistry 1:1000 - 1:5000, Immunocytochemistry/ Immunofluorescence 1:500 - 1:2500, Immunoprecipitation 1-4 ug/mg of lysate, Immunohistochemistry-Paraffin 1:1000 - 1:5000
Application Notes	Epitope retrieval with citrate buffer pH 6.0 is recommended for FFPE tissue sections. ICC/IF reactivity reported in (PMID: 27713178).

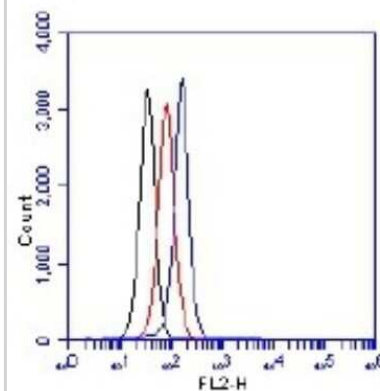


Images

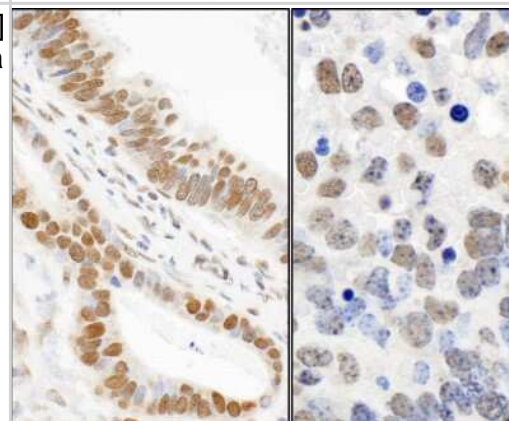
Western Blot: 53BP1 [p Ser25] Antibody [NB100-1803] - Samples: Whole cell lysate (30 ug or 15 ug for WB; 1 mg for IP, 20% of IP loaded) from 293T cells that were mock treated or treated with NCS (200 ng/ml; 1h). Affinity purified rabbit anti-Phospho 53BP1 antibody at 0.04 ug/ml (A) and 0.1 ug/ml (B) for WB. 53BP1 was immunoprecipitated using affinity purified rabbit anti-53BP1 Antibody (Catalog #NB100-304). Detection: Chemiluminescence with exposure times of 3 seconds (A and B). Bands appear at ~350 kDa as the observed molecular weight and the theoretical molecular weight is 214 kDa.



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) (Catalog #NB100-1803) in a 150 ul reaction. Isotype control (black), untreated (red), NCS treated (blue).



Immunohistochemistry-Paraffin: 53BP1 [p Ser25] Antibody [NB100-1803] - FFPE sections of human stomach carcinoma (left) and mouse teratoma (right). Antibody: Affinity purified rabbit anti- Phospho 53BP1 (S25) used at a dilution of 1:1,000 (1ug/ml). Detection: DAB.



Publications

Mehibel M, Xu Y, Li CG et al. Eliminating hypoxic tumor cells improves response to PARP inhibitors in homologous recombination-deficient cancer models *The Journal of clinical investigation* 2021-06-01 [PMID: 34060485] (WB, Human)

Howard SM, Ceppi I, Anand R et al. The internal region of CtIP negatively regulates DNA end resection *Nucleic Acids Res.* 2020-04-29 [PMID: 32347940] (WB, Human)

Ritter A, Friemel A, Kreis NN et al. Impact of Polo-like kinase 1 inhibitors on human adipose tissue-derived mesenchymal stem cells. *Oncotarget.* 2016-12-20 [PMID: 27713178] (ICC/IF, Human)

Sears CR, Cooney SA, Chin-Sinex H et al. DNA damage response (DDR) pathway engagement in cisplatin radiosensitization of non-small cell lung cancer. *DNA Repair (Amst)* 2016-04-01 [PMID: 26991853]

Yi YW, Park JS, Kwak SJ, Seong YS. Co-treatment with BEZ235 Enhances Sensitivity of BRCA1-negative Breast Cancer Cells to Olaparib *Anticancer Res.* 2015-07-01 [PMID: 26124328] (Human)

Harding SM, Bristow RG. Discordance between phosphorylation and recruitment of 53BP1 in response to DNA double-strand breaks. *Cell Cycle* 2012-04-01 [PMID: 22421153] (Human)

Pathania S, Bade S, Le Guillou M et al. BRCA1 haploinsufficiency for replication stress suppression in primary cells. *Nat Commun.* 2014-11-17 [PMID: 25400221]

Mirza S, Katafiasz BJ, Kumar R et al. Alteration/deficiency in activation-3 (Ada3) plays a critical role in maintaining genomic stability *Cell Cycle* 2012-11-15 [PMID: 23095635] (WB, Mouse)

Ota et al. *Hum.Mol.Genet.* 18 (14):2609-2621. 2009-01-01 [PMID: 19403560]

Kang Y, Lee J-H, Hoan NN et al. Protein Phosphatase 5 Regulates the Function of 53BP1 after Neocarzinostatin-induced DNA Damage. *J Biol Chem*;284(15):9845-9853. 2009-01-01 [PMID: 19176521]

Sakasai R, Tibbetts R. RNF8-dependent and RNF8-independent regulation of 53BP1 in response to DNA damage. *J Biol Chem* 2008-05-01 [PMID: 18337245]

Matsuoka S, Ballif BA, Smogorzewska A et al. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* 2007-05-25 [PMID: 17525332]

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Procedures

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

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

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

Reagents Required:

16% paraformaldehyde (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 Ml 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 10⁶ 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 20C.
5. Pour off media + PFA into dedicated PFA waste container.
6. Permeabilize cells by resuspending in ice cold 90% methanol to a final cell concentration of 1 X 10⁶ cells/ml.
7. Cells may be stored at -70 to -20C for up to a month.

Intracellular Staining:

1. Aliquot 1 ml (1 x 10⁶ cells) of cells in methanol for each tube/sample.
2. Spin at 250 x g for 5 minutes at 20C, 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.



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Products Related to NB100-1803

NB820-59453	Human Stomach Whole Tissue Lysate (Adult Whole Tumor)
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations

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