Product Datasheet

BRIP1/FANCJ Antibody
NB100-416

Unit Size: 0.1 ml

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

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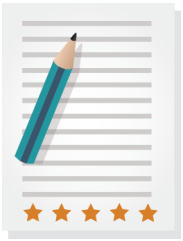
Updated 12/20/2023 v.20.1



NB100-416

BRIP1/FANCJ Antibody

Product Information	
Unit Size	0.1 ml
Concentration	This product is unpurified. The exact concentration of antibody is not quantifiable.
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.01% Sodium Azide
Isotype	IgG
Purity	Unpurified
Buffer	Whole antisera
Target Molecular Weight	130 kDa

Product Description	
Host	Rabbit
Gene ID	83990
Gene Symbol	BRIP1
Species	Human, Mouse
Immunogen	A synthetic peptide within the extreme C-terminus of human FANCJ. [Swiss-Prot# Q9BX63]

Product Application Details	
Applications	Western Blot, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Western Blot 1:500-1:200, Immunohistochemistry 1:10-1:500, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:200, Immunoblotting
Application Notes	Use in immunoblotting reported in scientific literature (PMID 25070891). The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

Publications

Alghoul E, Basbous J, Constantinou A An optogenetic proximity labeling approach to probe the composition of inducible biomolecular condensates in cultured cells STAR Protocols 2021-09-01 [PMID: 34377994] (IP)

Frattini C, Promonet A, Alghoul E, et al. TopBP1 assembles nuclear condensates to switch on ATR signaling Molecular cell 2021-01-16 [PMID: 33503405]

Brannvoll A, Xue X, Kwon Y et al. The ZGRF1 Helicase Promotes Recombinational Repair of Replication-Blocking DNA Damage in Human Cells Cell Rep 2020-07-07 [PMID: 32640219] (WB, Human)

Rossi F, Noren H, Sarria L, et al. SMC5/6 acts jointly with Fanconi anemia factors to support DNA repair and genome stability EMBO Rep. 2019-12-23 [PMID: 31867888]

Popp I, Punekar M, Telford N et al. Fanconi anemia with sun-sensitivity caused by a Xeroderma pigmentosum-associated missense mutation in XPF BMC Med. Genet. 2018-01-11 [PMID: 29325523] (WB, Human)

Bermudez-Hernandez K, Keegan S, Whelan DR et al. A Method for Quantifying Molecular Interactions Using Stochastic Modelling and Super-Resolution Microscopy Sci Rep. 2017-11-01 [PMID: 29093506] (Human)

Romick-Rosendale LE, Hoskins EE, Privette Vinnedge LM et al. Defects in the Fanconi anemia pathway in head and neck cancer cells stimulate tumor cell invasion through DNA-PK and Rac1 signaling. Clin. Cancer Res. 2015-11-24 [PMID: 26603260]

Aarts M, Bajrami I, Herrera-Abreu MT et al. Functional genetic screen identifies increased sensitivity to WEE1 inhibition in cells with defects in Fanconi Anaemia and HR pathways Mol. Cancer Ther. 2015-02-11 [PMID: 25673822] (WB, Human)

Vaz F, Hanenberg H, Schuster B et al. Mutation of the RAD51C gene in a Fanconi anemia-like disorder. Nat Genet. 2010-05-01 [PMID: 20400963] (WB, Human)

Knies K, Schuster B, Ameziane N et al. Genotyping of fanconi anemia patients by whole exome sequencing: advantages and challenges PLoS One 2012-01-01 [PMID: 23285130] (WB, Human)

Sakasai R, Sakai A, Iimori M et al. CtIP- and ATR-dependent FANCJ phosphorylation in response to DNA strand breaks mediated by DNA replication. Genes Cells 2012-12-01 [PMID: 23157317] (WB, Human)

Cantor, S et al. BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. Cell;105(1):149-60. 2001-04-06 [PMID: 11301010] (WB, IP, Human)

More publications at http://www.novusbio.com/NB100-416

Procedures

Chromatin Immunoprecipitation (ChIP)
protocol specific for FANCJ Antibody (NB100-416)

1) Grow cells in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum to 95% confluence.

2) Cross-linked cells with 1% formaldehyde at room temperature for 10 minutes.

3) Rinse with ice-cold PBS twice and collect into 100 mM Tris-HCl (pH 9.4) and 10 mM DTT.

4) Incubate for 15 minutes at 30C and centrifuge for 5 minutes at 2,000 rpm.

5) Wash cells sequentially with:
a. 1.0 ml ice-cold PBS
b. Buffer I [0.25% Triton X-100, 10 mM EDTA, 0.5mM EGTA, 10 mM HEPES, pH 6.5)
c. Buffer II [200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 10 mM HEPES, pH 6.5
d. Centrifuge for 5 minutes.

6) Resuspend cells in 0.5 mL of Lysis Buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.1, 1X protease inhibitor cocktail (Roche Molecular Biochemicals, Indianapolis, IN) and sonicate 3 times for 10 seconds each at maximal input (Fisher Sonic Dismembrantor, Model 300).

7) Centrifuge for 10 minutes.

8) Collect supernatants and dilute 1:3 in Dilution Buffer (1% Triton X-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl, pH 8.1) followed by immunoclearing with 20 mg sheared salmon sperm DNA, 2 ml pre-immune serum or mouse whole IgG, and protein A-sepharose (50-50 slurry in 10 mM Tris-HCl, pH 8.1, 1 mM EDTA) for 2 hours at 4C.

9) Immunoprecipitation is performed for 6 hours or overnight at 4C with specific antibodies.

10) After IP add 45 ml protein A-sepharose, 10 mg of salmon sperm DNA. Continue IP for an additional hour.

11) Sepharose beads are washed sequentially in:
a. TSE I [0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 150 mM NaCl]
b. TSE II [0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1; 500 mM NaCl]
c. TSE III [0.25 M LiCl, 1% NP-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, pH 8.1]
d. Beads are then washed three times in TE buffer and extracted three times in 1% SDS, 0.1 M $NaHCO_3$.

12) Eluates (50 ml) are pooled and heated at 65C for 6 hours or overnight to reverse the formaldehyde cross-links.

13) DNA fragments are purified with a DNA purification kit (QIAquick Spin Kit, Qiagen, CA) and amplified with PCR.

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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.

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