Product Datasheet

FANCD2 Antibody
NB100-182SS

Unit Size: 0.025 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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NB100-182SS

FANCD2 Antibody

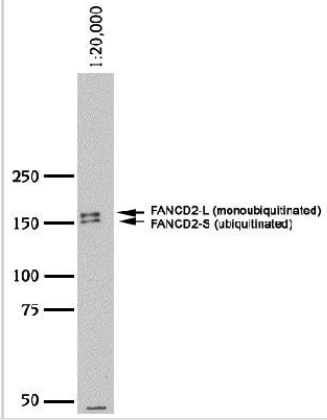
Product Information	
Unit Size	0.025 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Purity	Affinity purified
Buffer	Tris-glycine, 150mM NaCl, pH7.5

Product Description	
Host	Rabbit
Gene ID	2177
Gene Symbol	FANCD2
Species	Human, Mouse, Primate
Species Reactivity	NB 100-182 reacts with human and mouse FANCD2.
Specificity/Sensitivity	This antibody is specific for FANCD2.
Immunogen	Human FANCD2 fusion protein (N-terminal fragment). [Swiss-Prot #Q9BXW9]

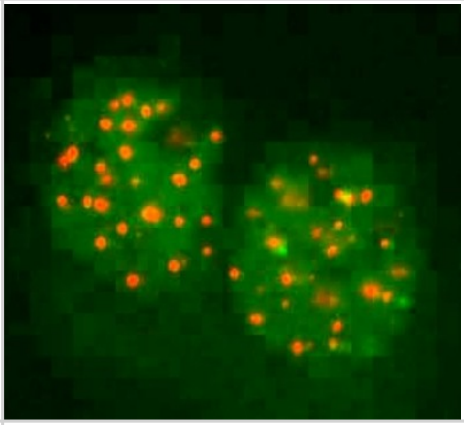
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Immunocytochemistry/Immunofluorescence 1:200-1:500, Immunohistochemistry 2.5-5.0 ug/ml, Immunohistochemistry-Paraffin 2.5-5.0 ug/ml, Immunoprecipitation 1:10-1:500, Western Blot 1:10000-1:20000, Simple Western 1:25
Application Notes	By Western blot, this antibody should recognize a band at ~166 kDa (post-translationally modified form). Additional bands may be seen at lower molecular weights. For immunofluorescence it has been tested in human MMC and IR treated MEF cells. This antibody may also be used for Immunoprecipitation and Immunohistochemistry-Paraffin In Simple Western only 10-15 uL of the recommended dilution is used per data point.

Images

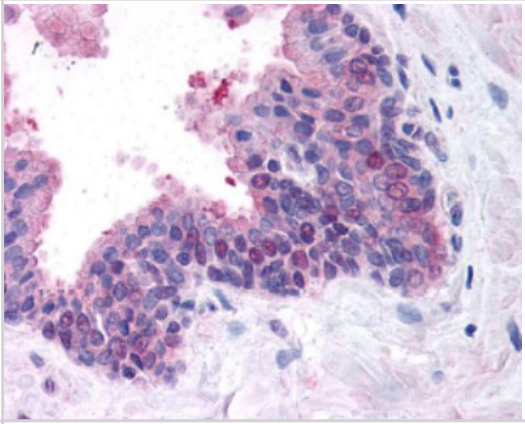
Western Blot: FANCD2 Antibody [NB100-182] - FANCD2 in HeLa WCE using NB 100-182 (lot C).



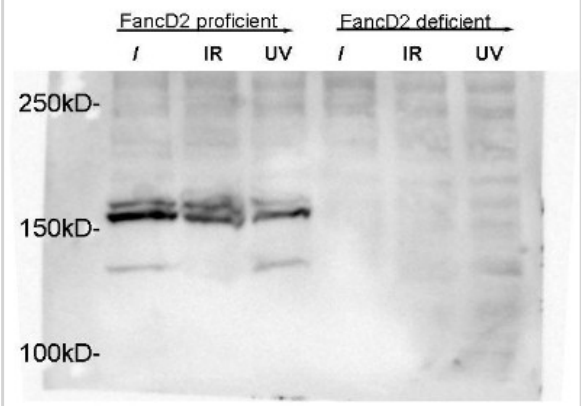
Immunocytochemistry/Immunofluorescence: FANCD2 Antibody [NB100-182] - FancD2 colocalizes in vivo with another protein in U2OS cells after cell exposure to IR. Proliferating U2OS cells were exposed to 10 Gy of IR and double -color immunofluorescence staining was performed after 8 h. Images were captured in a Kodak digital image system on a Leica fluorescence microscope.



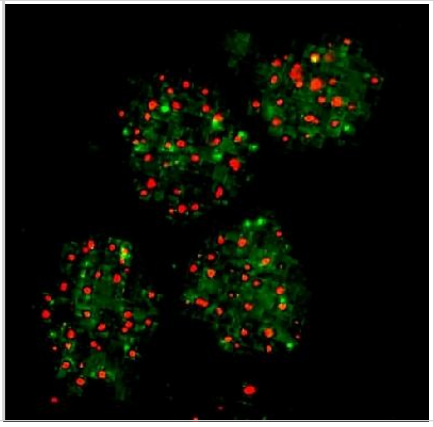
Immunohistochemistry: FANCD2 Antibody [NB100-182] - Staining of human prostate, glandular epithelium.



Western Blot: FANCD2 Antibody [NB100-182] - Analysis of FANCD2 in human PD20 cells. Image courtesy of anonymous customer review



Immunocytochemistry/Immunofluorescence: FANCD2 Antibody [NB100-182] - FancD2 colocalizes in vivo with another protein in SiHa cells after cell exposure to IR. Proliferating SiHa cells were exposed to 10 Gy of IR and double -color immunofluorescence staining was performed after 8 h. Images were captured in a Kodak digital image system on a Leica fluorescence microscope.



Simple Western: FANCD2 Antibody [NB100-182] - Simple Western lane view shows a specific band for FANCD2 in 0.1 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

Publications

Duan W, Gao L, Aguila B et al. Fanconi anemia repair pathway dysfunction, a potential therapeutic target in lung cancer. Front Oncol. 2015 Jan 08 [PMID: 25566506] (WB, IHC-P, Human)

Details:
The paraffin-embedded human lung tumor tissue sections stained with FANCD2 antibody and analyzed by FATSI method. The FANCD2 antibody was also used for WB analysis on lung cancer cells.

Stoepker C, Faramarz A, Rooimans MA, van Mil SE. DNA helicases FANCM and DDX11 are determinants of PARP inhibitor sensitivity. DNA Repair. 2014 Dec 24 [PMID: 25583207] (ICC/IF, Human)

Details:
FANCD2 antibody used for ICC-IF in experiments involving head and neck tumor cell lines (VU-SCC-1131 (FANCC-deficient), VU-SCC-1365 (FANCA-deficient) and VU-SCC-1604 (FANCL-deficient)) derived from FA patients (Fig. 5D).

Bursomanno S, Beli P, Khan Am, Minocherhomji S. Proteome-wide analysis of SUMO2 targets in response to pathological DnA replication stress in human cells DnA Repair et al. 2014 Nov 25 [PMID: 25497329] (WB, Human)

Details:
FAnCD2 antibody used for WB on U2OS-Strep-HA-SUMO2 cells that were either untreated, treated with HU alone (HU-release), or HU and APH (HU-APH) conditions (Fig. 5B).

Chlon TM, Hoskins EE, Mayhew CN et al. High risk HPV E6 protein promotes reprogramming of Fanconi Anemia patient cells through repression of p53 but does not allow for sustained growth of iPSC. J. Virol. 2014 Jul 16 [PMID: 25031356] (WB, Human)

Details:
FANCD2 antibody used for WB in FA patients-derived cell (FIG 2B) and on induced pluripotent stem cells/iPSC lines as well as parental keratinocytes from patients FA-A2 to -4 treated with 1 mM hydroxyurea (FIG 5J).

Panneerselvam J, Pickering A, Han B et al. Basal level of FANCD2 monoubiquitination is required for the maintenance of a sufficient number of licensed-replication origins to fire at a normal rate. Oncotarget 2014 Jul 15 [PMID: 24658369] (WB, Human)

Details:
FANCD2 antibody used for WB on several experiments involving CRL-1790 and HEK293T cells. Antibody also used in stably transfected PD20 cells.

Huard CC, Tremblay CS, Magron A, Carreau M. The Fanconi anemia pathway has a dual function in Dickkopf-1 transcriptional repression. Proc. Natl. Acad. Sci. U.S.A. 2014 Feb 12 [PMID: 24469828] (WB, Human)

Boisvert RA, Rego MA, Azzinaro PA et al. Coordinate Nuclear Targeting of the FANCD2 and FANCI Proteins via a FANCD2 Nuclear Localization Signal. PLoS One. 2013 Nov 21 [PMID: 24278431] (WB, ICC/IF, Human)

Figueiredo N, Chora A, Raquel H et al. Anthracyclines Induce DNA Damage Response-Mediated Protection against Severe Sepsis. Immunity. 2013 Nov 14 [PMID: 24184056] (WB, Human)

Wha Jun D, Hwang M, Kim HJ et al. Ouabain, a Cardiac Glycoside, Inhibits the Fanconi Anemia/BRCA Pathway Activated by DNA Interstrand Cross-Linking Agents. PLoS One. 2013 Oct 4 [PMID: 24124520] (WB, Human)

Wang Y, Han X, Wu F et al. Structure analysis of FAAP24 reveals single-stranded DNA-binding activity and domain functions in DNA damage response. Cell Res. 2013 Oct [PMID: 23999858] (WB, Human)

Coulthard R, Deans AJ, Swuec P et al. Architecture and DNA Recognition Elements of the Fanconi Anemia FANCM-FAAP24 Complex. Structure. 2013 Sep 3 [PMID: 23932590] (WB, Human)

Pfaffle HN, Wang M, Gheorghiu L et al. EGFR activating mutations correlate with a Fanconi anemia-like cellular phenotype that includes PARP inhibitor sensitivity. Cancer Res. 2013 Aug 21 [PMID: 23966292] (ICC/IF, Human)

More publications at http://www.novusbio.com/NB100-182

Procedures

Western Blot Protocol for FANCD2 Antibody (NB100-182)

Western Blot

1. Proteins are separated on a 7.5% SDS-PAGE gel.
2. Following the protein transfer, the membrane is blocked with TBST + 3% milk.
3. Anti-FANCD2 [cat# NB 100-182] is diluted in blocking buffer and incubated for 1 hour at room temperature, gently shaking.
4. The membrane is then washed, 3 times with TBST, 5 minutes each.
5. Secondary antibody is incubated for 1 hour at room temperature, gently shaking.
6. The membrane is then washed, 3 times with TBST, 5 minutes each.
7. Membrane is developed using ECL reagents. Visualization after a 30 second -2 minute exposure.

NOTE: HeLa whole cell extracts [cat# NB 800-PC1] were used as positive control for this antibody

Immunoprecipitation

4ul for 1 mg of whole cell extract prepared by lysis in either standard RIPA buffer or a mild buffer composed of 10 mM Tris/HCl pH 8, 150 mM NaCl, 2 mM MgCl2, 0.1% Triton X-100 + inhibitors.

Immunohistochemistry - 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.

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.
S. 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 Celcius 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 1-2; 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).

Novus Biologicals USA
8100 Southpark Way, A-8
Littleton, CO 80120
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
novus@novusbio.com

Novus Biologicals Canada
461 North Service Road West, Unit B37
Oakville, ON L6M 2V5
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada@novusbio.com

Novus Biologicals Europe
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: technical@novusbio.com
Orders: orders@novusbio.com
General: novus@novusbio.com

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 guarantee, please visit www.novusbio.com/guarantee.