Product Datasheet

NOD2 Antibody
NB500-253

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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Publications: 5

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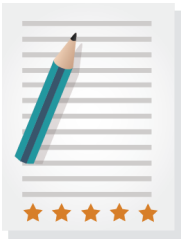
Updated 7/27/2022 v.20.1



NB500-253

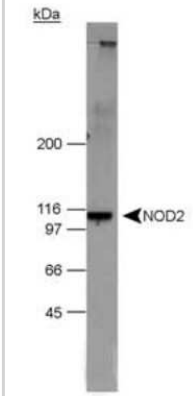
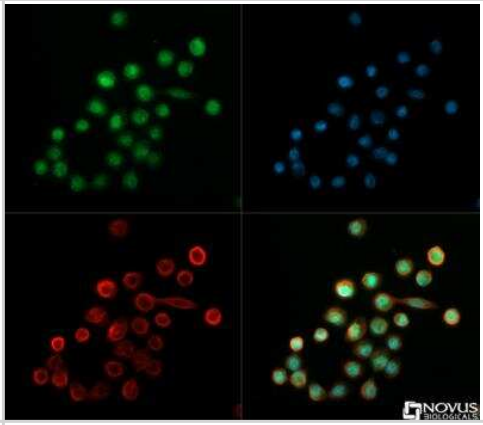
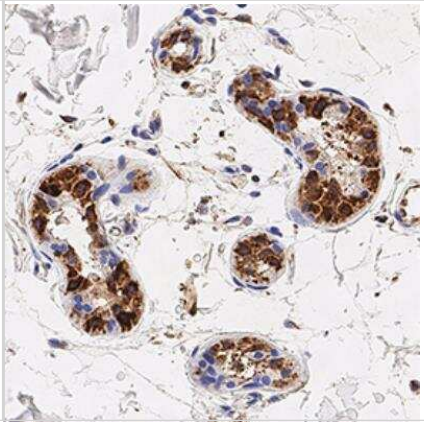
NOD2 Antibody

Product Information	
Unit Size	0.1 ml
Concentration	0.87 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	Tris-Glycine, 0.15 M NaCl
Target Molecular Weight	110 kDa

Product Description	
Host	Rabbit
Gene ID	64127
Gene Symbol	NOD2
Species	Human
Immunogen	A synthetic peptide made to the C-terminal region of human NOD2 (between residues 1000-1040). [UniProt# Q9HC29]

Product Application Details	
Applications	Western Blot, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Western Blot 0.5 - 2 ug/mL, Immunohistochemistry 1:250, Immunocytochemistry/Immunofluorescence 1:50 - 1:100, Immunoprecipitation, Immunohistochemistry-Paraffin 1:250
Application Notes	This NOD2 antibody has been tested for Western blot on NOD2 transfected 293T lysates where a band is seen at ~110 kDa. We did not see positive results probing endogenous protein using HT29 lysates. 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. Use in Immunoprecipitation reported in scientific literature (PMID:30709874).

Images

Western Blot: NOD2 Antibody [NB500-253] - Detection of NOD2 in 20 ug of NOD2 transfected 293T cell lysate using NB 500-253. ECL detection in 15 seconds.	
Immunocytochemistry/Immunofluorescence: NOD2 Antibody [NB500-253] - NOD2 antibody was tested in HT-29 cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).	
Immunohistochemistry-Paraffin: NOD2 Antibody [NB500-253] - Analysis of FFPE human skin using NOD2 antibody at 1:250 on a Bond Rx autostainer (Leica Biosystems). The assay involved 20 minutes of heat induced antigen retrieval (HIER) using 10 mM sodium citrate buffer (pH 6.0) and endogenous peroxidase quenching with peroxide block. The sections were incubated with primary antibody for 30 minutes and Bond Polymer Refine Detection (Leica Biosystems) with DAB was used for signal development followed by counterstaining with hematoxylin. Whole slide scanning and capturing of representative images was performed using Aperio AT2 (Leica Biosystems). Cytoplasmic staining of NOD2 was observed. Staining was performed by Histowiz.	

Publications

Ahn M Y, Kang J K et al. Expression of nucleotide-binding oligomerization domain 1 and 2 in oral lichen planus. J Dent Sci 2020-01-03 [PMID: 32256993] (IF/IHC, Human)

Lipinski S, Petersen BS, Barann M et al. Missense variants in NOX1 and p22phox in a case of very-early-onset inflammatory bowel disease are functionally linked to NOD2 Cold Spring Harb Mol Case Stud. 2017-11-07 [PMID: 30709874] (IP, WB, Human)

Lappas M. NOD1 and NOD2 Regulate Proinflammatory and Prolabor Mediators in Human Fetal Membranes and Myometrium via Nuclear Factor-Kappa B. Biol Reprod 2013-07-18 [PMID: 23740944]

Lipinski S, Grabe N, Jacobs et al. RNAi screening identifies mediators of NOD2 signaling: Implications for spatial specificity of MDP recognition Proc Natl Acad Sci U S A 2012-12-03 [PMID: 23213202] (IF/IHC, ICC/IF, WB, Human)

Till A et al. A role for membrane-bound CD147 in NOD2-mediated recognition of bacterial cytoinvasion. J Cell Sci;121 (Pt 4):487-95. 2008-02-15 [PMID: 18256385] (ICC/IF, Human)

Procedures

Western Blot protocol for NOD2 Antibody (NB500-253)
NOD2 Antibody: https://www.novusbio.com/products/nod2-antibody_nb500-253
Western Blot Protocol

1. Perform SDS-PAGE (3-8%) on samples to be analyzed, loading 20 ug of total transfected protein per lane.

2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.

3. Stain the blot using ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.

4. Rinse the blot in TBS for approximately 5 minutes.

5. Block the membrane using 5% non-fat dry milk in TBS for 1 hour.

6. Dilute the rabbit anti-NOD2 primary antibody (NB 500-253) in blocking buffer and incubate 2 hours at room temperature.

7. Wash the membrane in water for 5 minutes and apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) and incubate 1 hour at room temperature.

8. Wash the blot in TBS containing 0.05-0.1% Tween-20 for 10-20 minutes.

9. Wash the blot in type I water for an additional 10-20 minutes (this step can be repeated as required to reduce background).

10. Apply the detection reagent of choice in accordance with the manufacturer's instructions (Amersham's ECL is the standard reagent used at Novus Biologicals).

Note: Tween-20 can be added to the blocking buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

Immunocytochemistry/Immunofluorescence protocol for NOD2 Antibody (NB500-253)
NOD2 Antibody: https://www.novusbio.com/products/nod2-antibody_nb500-253
Immunocytochemistry Protocol

Culture cells to appropriate density on suitable glass coverslips 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 5-10 minutes.
2. Remove the formalin and add 0.5% Triton-X 100 in TBS to permeabilize the cells. Incubate for 5-10 minutes.
3. Remove the permeabilization buffer and add wash buffer (i.e. PBS or PBS with 0.1% Tween-20). Be sure to not let the specimen dry out. Gently wash three times for 10 minutes.
4. Alternatively, cells can be fixed with -20C methanol for 10 min at room temperature. Remove the methanol and rehydrate in PBS for 10 min before proceeding.
5. To block nonspecific antibody binding incubate in 10% normal goat serum for 1 hour at room temperature.
6. Add primary antibody at appropriate dilution and incubate at room temperature for 1 hour or at 4 degrees C overnight.
7. Remove primary antibody and replace with wash buffer. Gently wash three times for 10 minutes.
8. Add secondary antibody at the appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove antibody and replace with wash buffer. Gently wash three times for 10 minutes.
10. Nuclei can be staining with 4',6' diamino phenylindole (DAPI) at 0.1 ug/ml, or coverslips can be directly mounted in media containing DAPI.
11. Cells can now be viewed with a fluorescence microscope.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow proper laboratory procedures for the disposal of formalin.

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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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