

Product Datasheet

TLR4 Antibody - BSA Free NB100-56581

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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NB100-56581

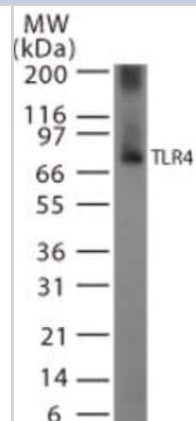
TLR4 Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.12 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	PBS, 30% Glycerol
Target Molecular Weight	95.7 kDa
Product Description	
Host	Rabbit
Gene ID	7099
Gene Symbol	TLR4
Species	Human, Mouse, Rat
Reactivity Notes	Rat reactivity reported in scientific literature (PMID: 23744481).
Immunogen	This TLR4 antibody was developed against a synthetic peptide made to an internal portion of the mouse TLR4 protein (between residues 400-450) [Q9QUK6].
Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Proximity Ligation Assay
Recommended Dilutions	Western Blot 1 - 3 ug/mL, Flow Cytometry 1:10 - 1:1000, Immunohistochemistry 1:50, Immunocytochemistry/ Immunofluorescence 1:25 - 1:75, Immunohistochemistry-Paraffin 1:50, Immunohistochemistry-Frozen reported in scientific literature (PMID 24128422), Immunoblotting reported in scientific literature (PMID 23744481), Proximity Ligation Assay reported in scientific literature (PMID 30579042)

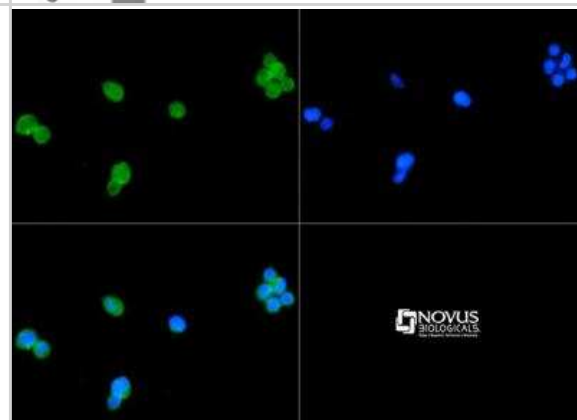


Images

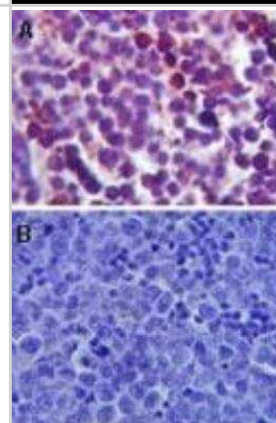
Western Blot: TLR4 Antibody [NB100-56581] - Analysis of TLR4 using NB100-56581 at 2 ug/mL on partial recombinant mouse TLR4 protein.



Immunocytochemistry/Immunofluorescence: TLR4 Antibody [NB100-56581] - TLR4 antibody was tested in PC-12 cells with FITC (green). Nuclei were counterstained with DAPI (blue).



Immunohistochemistry-Paraffin: TLR4 Antibody [NB100-56581] - Analysis of TLR4 in FFPE mouse spleen tissue using NB100-56581 (A) and mouse isotype control (B) at 5 mg/mL.



Publications

Smith P, Godde N, Rubio S et al. Modification of primary amines to higher order amines reduces in vivo hematological and immunotoxicity of cationic nanocarriers through TLR4 and complement pathways *Biomaterials* 2019-10-05 [PMID: 31585233]

Takeo M, Nishio A, Masuda M Et al. Repeated Stimulation of Toll-Like Receptor 2 and Dectin-1 Induces Chronic Pancreatitis in Mice Through the Participation of Acquired Immunity *Digestive diseases and sciences* 2021-08-23 [PMID: 34424458]

Kopiasz Ł, Dziendzikowska K, Gajewska M, et al. Effects of Dietary Oat Beta-Glucans on Colon Apoptosis and Autophagy through TLRs and Dectin-1 Signaling Pathways-Crohn's Disease Model Study *Nutrients* 2021-01-22 [PMID: 33499397] (IF/IHC, Rat)

Riehl TE, Alvarado D, Ee X et al. Hyaluronic acid promotes Lgr5+ stem cell proliferation and crypt fission through TLR4 and PGE2 transactivation of EGFR *Am. J. Physiol. Gastrointest. Liver Physiol.* 2020-06-15 [PMID: 32538139]

Arnaboldi F, Sommariva M, Opizzi E et al. Expression of Toll-like receptors 4 and 7 in murine peripheral nervous system development *Ann. Anat.* 2020-05-04 [PMID: 32380196] (IHC-P, Mouse)

Blanchard EL, Loomis KH, Bhosle SM et al. Proximity ligation assays for in situ detection of innate immune activation: focus on in vitro transcribed mRNA. *Mol Ther Nucleic Acids.* [PMID: 30579042] (PLA, Mouse)

Jia R, Jia N, Yang F et al. Modification of primary amines to higher order amines reduces in vivo hematological and immunotoxicity of cationic nanocarriers through TLR4 and complement pathways *bioRxiv* (FLOW, Mouse)

Burgueno, JF Understanding the role of Toll-like receptors in the lower gastrointestinal tract. Thesis. 2014-01-01 (IHC-P, Mouse)

Details:
Colon, Figs 1-3

Flores-Espinosa P, Pineda-Torres M, Vega-Sanchez R et al. Progesterone Elicits an Inhibitory Effect upon LPS-Induced Innate Immune Response in Pre-Labor Human Amniotic Epithelium. *Am J Reprod Immunol.* 2013-10-16 [PMID: 24128422] (ICC/IF, IHC-Fr, Human)

Cammarota R, Bertolini V, Pennesi G et al. The tumor microenvironment of colorectal cancer: stromal TLR-4 expression as a potential prognostic marker. *J Transl Med.* 2010-11-08 [PMID: 21059221] (IHC-P, Mouse)

Li X, Kroin JS, Kc R et al. Altered spinal microRNA-146a and the microRNA-183 cluster contribute to osteoarthritic pain in knee joints. *J Bone Miner Res* 2013-06-06 [PMID: 23744481]

Lu Z, Li Y, Samuvel DJ, Jin J et al. MD-2 Is Involved in the Stimulation of MMP-1 Expression by IFN γ and High Glucose in Mononuclear Cells - A Potential Role of MD-2 in TLR4-Independent Signaling. *Immunology* 2013-06-26 [PMID: 23800176] (FLOW, Human)

More publications at <http://www.novusbio.com/NB100-56581>

Procedures

Western Blot protocol for TLR4 Antibody (NB100-56581)

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

****Note:** Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

Immunohistochemistry-Paraffin protocol for TLR4 Antibody (NB100-56581)

Antigen Unmasking:

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

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Immunocytochemistry/Immunofluorescence protocol for TLR4 Antibody (NB100-56581)

Culture cells to appropriate density 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 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

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Products Related to NB100-56581

NBP2-25295-1.0mg	LPS from E. Coli, TLR4 ligand
NBP2-26244	TLR4 Inhibitor Peptide Set
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

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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