Product Datasheet

TLR4 Antibody
NB100-56581SS

Unit Size: 0.025 ml

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

Reviews: 1 Publications: 9

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB100-56581

Updated 6/15/2014 v.20.1

NB100-56581SS

TLR4 Antibody

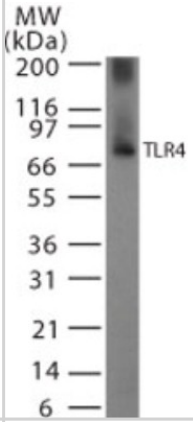
Product Information	
Unit Size	0.025 ml
Concentration	1.12 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Purity	Immunogen affinity purified
Buffer	PBS, 30% glycerol
Target Molecular Weight	75 kDa

Product Description	
Host	Rabbit
Gene ID	7099
Gene Symbol	TLR4
Species	Human, Mouse
Species Reactivity	Human and mouse.
Immunogen	A synthetic peptide made to an internal portion of the mouse TLR4 protein (between residues 400-450) [NP_612564]

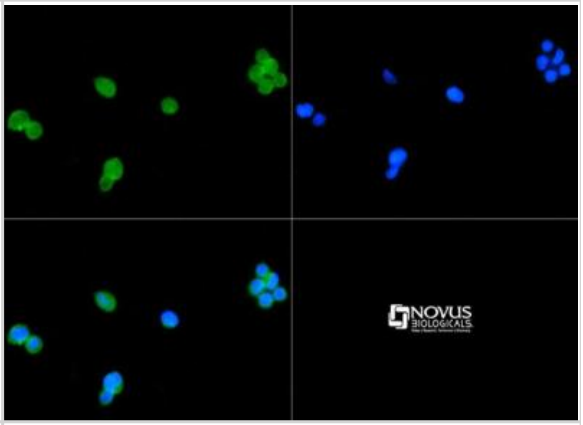
Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Flow Cytometry 1:10-1:1000, Immunocytochemistry/Immunofluorescence 1:25-1:75, Immunohistochemistry 1:50, Immunohistochemistry-Paraffin 1:50, Western Blot 1-3 ug/ml
Application Notes	This TLR4 antibody is useful for Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin and Western Blot.

Images

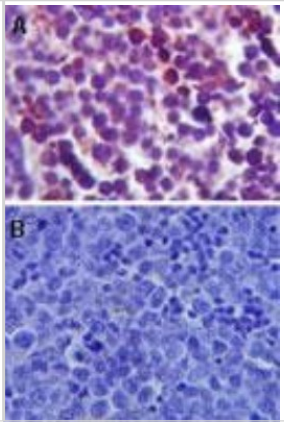
Western Blot: TLR4 Antibody [NB100-56581] - Analysis of TLR4 using NB 100-56581 at 2 ug/ml on partial recombinant mouse TLR4 protein.



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 formalin-fixed, paraffin-embedded mouse spleen tissue using NB100-56581 (A) and mouse isotype control (B) at 5 mg/ml.



Publications

Burgueno, JF Understanding the role of Toll-like receptors in the lower gastrointestinal tract. Thesis. 2014 (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 Oct 16 [PMID: 24128422] (IHC-Fr, ICC/IF, 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 Nov 8 [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 Jun 6 [PMID: 23744481]

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

El Shikh MEM, RM El Sayed, Y Wu, AK Szakal, JG Tew. TLR4 on follicular dendritic cells: An activation pathway that promotes accessory activity. J Immunol 179:4444-4450. 2007 [PMID: 17878340]

Herber DL, JL Maloney, LM Roth, MJ Freeman, D Morgan MN Gordon. Diverse microglial responses after intrahippocampal admnistration of lipopolysaccharide. GLIA 53:382-391. 2006 [PMID: 16288481] (IHC, Mouse)

Barajon I, Serrao G, Arnaboldi F et al. Toll-like receptors 3, 4, and 7 are expressed in the enteric nervous system and dorsal root ganglia. J Histochem Cytochem;57(11):1013-23. 2009 Nov. [PMID: 19546475] (IHC, WB, Mouse)

Carrithers M et al. Enhanced susceptibility to endotoxic shock and impaired STAT3 signaling in CD31-deficient mice. Am J Pathol;166(1):185-96. 2005 Jan. [PMID: 15632011] (WB, FLOW, Mouse)

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.

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

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 1:25,0000 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.

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

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.