Product Datasheet

TLR4 Antibody - BSA Free

NBP1-78427

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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NBP1-78427

TLR4 Antibody - BSA Free

Product Information

Unit Size	0.1 ml
Concentration	1.14 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

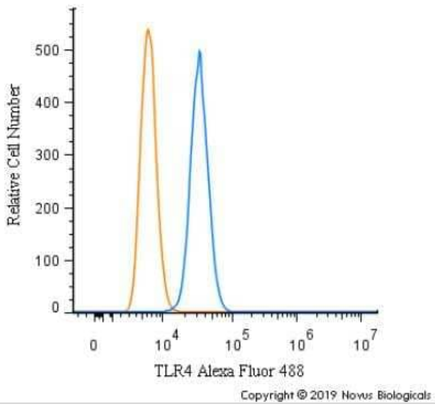
Host	Rabbit
Gene ID	7099
Gene Symbol	TLR4
Species	Human, Mouse, Rat
Immunogen	This TLR4 antibody was developed against a synthetic peptide made to the C-terminal portion of the human TLR4 protein (between residues 650-710) [Uniprot: O00206].

Product Application Details

Applications	Western Blot, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:1000, Flow Cytometry 2 - 5 ug / million cells, Immunohistochemistry 1:50, Immunocytochemistry/ Immunofluorescence 1:50, Immunohistochemistry-Paraffin 1:50
Application Notes	Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.

Images

Flow Cytometry: TLR4 Antibody [NBP1-78427] - An intracellular stain was performed on RH-30 cells with NBP1-78427AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.



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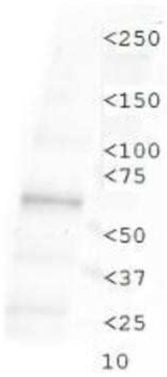
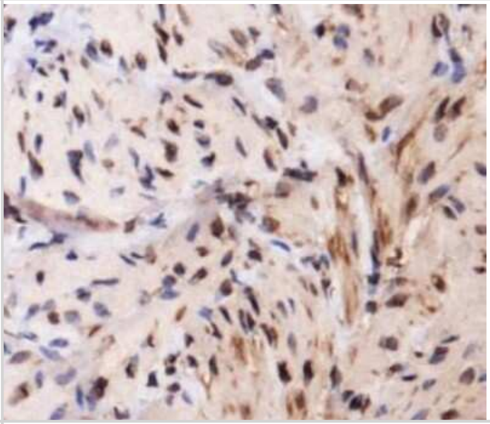
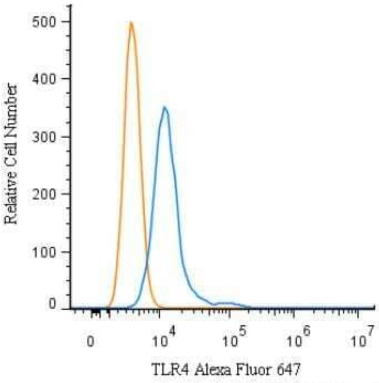
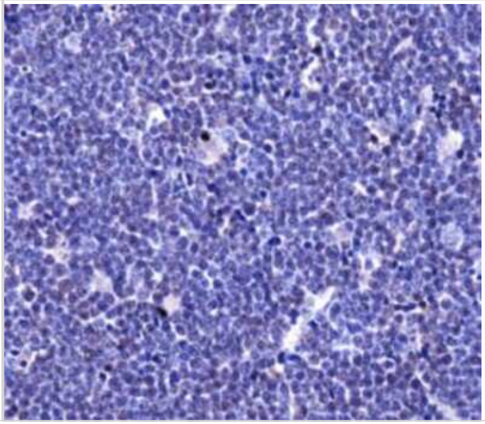
Western Blot: TLR4 Antibody [NBP1-78427] - Analysis of TLR4 in Raw 264.7 cell lysate.	
Immunohistochemistry: TLR4 Antibody [NBP1-78427] - Analysis of TLR4 in mouse smooth muscle using DAB wtih hematoxylin counterstain.	
Flow Cytometry: TLR4 Antibody [NBP1-78427] - An intracellular stain was performed on RH-30 cells with TLR4 Antibody NBP1-78427AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.	 Copyright © 2019 Novus Biologicals
Immunohistochemistry: TLR4 Antibody [NBP1-78427] - Analysis of TLR4 in mouse spleen using DAB with hematoxylin counterstain.	

Publications

Shim KH, Ha S, Choung JS et al. Therapeutic Effect of Erythropoietin on Alzheimer's Disease by Activating the Serotonin Pathway International Journal of Molecular Sciences 2022-07-24 [PMID: 35897720] (Western Blot)

Son M, Oh S, Jang JT et al. Attenuating Effects of Pyrogallol-Phloroglucinol-6,6-Bieckol on Vascular Smooth Muscle Cell Phenotype Changes to Osteoblastic Cells and Vascular Calcification Induced by High Fat Diet Nutrients 2020-09-11 [PMID: 32932908] (IHC-P, Mouse)

Aurelian L, Warnock KT, Balan I et al. TLR4 signaling in VTA dopaminergic neurons regulates impulsivity through tyrosine hydroxylase modulation. Transl Psychiatry. 2016-05-18 [PMID: 27187237] (ICC/IF, WB, Rat)

Trune DR, Kempton B, Hausman FA et al. Correlative mRNA and protein expression of middle and inner ear inflammatory cytokines during mouse acute otitis media Hear. Res. 2015-01-01 [PMID: 25922207] (IHC-P, Mouse)

Procedures

Western Blot protocol for TLR4 Antibody (NBP1-78427)

TLR4 Antibody:
Western Blot Protocol

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 (NBP1-78427)

TLR4 Antibody:
Immunohistochemistry-Paraffin Embedded Sections

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 (NBP1-78427)

TLR4 Antibody:
Immunocytochemistry Protocol

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.

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Products Related to NBP1-78427

NBP1-30157	Raw 264.7 Whole Cell Lysate
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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