Product Datasheet

TLR4 Antibody (76B357.1) - BSA Free
NB100-56566

Unit Size: 0.1 mg

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

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# NB100-56566

TLR4 Antibody (76B357.1) - BSA Free

## Product Information

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<th>Parameter</th>
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<td>Unit Size</td>
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<td>Concentration</td>
<td>1.0 mg/ml</td>
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<td>Storage</td>
<td>Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.</td>
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<tr>
<td>Clonality</td>
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<td>Clone</td>
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<td>Preservative</td>
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<td>Isotype</td>
<td>IgG2b Kappa</td>
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<td>Buffer</td>
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<td>Target Molecular Weight</td>
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## Product Description

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<td>Gene ID</td>
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<td>Gene Symbol</td>
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<tr>
<td>Species</td>
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<td>Immunogen</td>
<td>This TLR4 Antibody (76B357.1) was developed against a portion of amino acids 100-200 of human TLR4 (NP_612564).</td>
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## Product Application Details

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<th>Applications</th>
<th>Western Blot, Dot Blot, ELISA, Flow Cytometry, Flow (Cell Surface), Flow (Intracellular), Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, In vitro assay, SDS-Page, Block/Neutralize, Chromatin Immunoprecipitation (ChIP), CyTOF-ready, Dual RNAscope ISH-IHC, ELISA Capture (Matched Antibody Pair), Knockdown Validated, Knockout Validated</th>
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<td>Recommended Dilutions</td>
<td>Western Blot 1-3 ug/ml, Flow Cytometry, ELISA, Immunohistochemistry 1:10-1:500, Immunocytochemistry/Immunofluorescence 1:10-1:500, Immunohistochemistry-Paraffin 5 ug/ml, Immunohistochemistry-Frozen 1:500, In vitro assay, Dot Blot, SDS-Page reported in scientific literature (PMID 33166339), Flow (Cell Surface), Flow (Intracellular), ELISA Capture (Matched Antibody Pair) 0.5 ug, Chromatin Immunoprecipitation (ChIP) 1:10-1:500, CyTOF-ready, Knockout Validated, Knockdown Validated, Block/Neutralize, Dual RNAscope ISH-IHC</td>
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Images

Dual RNAscope ISH-IHC: TLR4 Antibody (76B357.1) [NB100-56566] - FFPE tissue sections of human tonsil were probed for TLR4 mRNA (ACD RNAscope Probe, ACD catalog # 311281; Fast Red chromogen, ACD catalog # 322750). Adjacent tissue section was processed for immunohistochemistry using Mouse Monoclonal (Novus Biologicals catalog # NB100-56566) at 5ug/mL with 1 hour incubation at room temperature followed by incubation with anti-mouse IgG VisUCyte HRP Polymer Antibody (Catalog # VC001) and DAB chromogen (yellow-brown). Tissue was counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes.

Flow Cytometry: TLR4 Antibody (76B357.1) [NB100-56566] - Analysis using the Alexa Fluor (R) 647 conjugate of NBP2-27149. TLR4 expression on monocytes from human peripheral blood. PBMC were stained in a 2 color flow test, with CD14 PE version of this antibody and 1 ug of either isotype control (left) or TLR4-Alexa Fluor 647 (right). PPI negative, CD14+ cells were gated for analysis.

Immunohistochemistry: TLR4 Antibody (76B357.1) [NB100-56566] - Pericryptal Myofibroblasts are Responsible for Increased TLR4 Expression in a Subset of CRCs. Double-stained immunofluorescence for TLR4 (green) and vimentin (red) in normal (I), adenoma (II), and colon adenocarcinoma (III) (10x). In the stromal compartment of CRCs, immunofluorescent staining for TLR4 localized to the pericryptal myofibroblasts in a subset of samples. Image collected and cropped by CiteAb from the following publication (http://www.jeccr.com/content/33/1/45), licensed under a CC-BY license.

Western Blot: TLR4 Antibody (76B357.1) [NB100-56566] - Analysis using 2 ug/mL on (A) human intestine and 6 ug/mL on (B) mouse intestine and (C) rat intestine lysate.
Immunohistochemistry: TLR4 Antibody (76B357.1) [NB100-56566] - Pericryptal Myofibroblasts are Responsible for Increased TLR4 Expression in a Subset of CRCs. IHC staining of colon adenocarcinoma for TLR4, vimentin, and alpha-SMA (40x). Staining co-localizes to the pericryptal space, confirming the signal arises from pericryptal myofibroblasts. Image collected and cropped by CiteAb from the following publication (http://www.jeccr.com/content/33/1/45), licensed under a CC-BY license.

Flow (Intracellular): TLR4 Antibody (76B357.1) [NB100-56566] - Analysis using PE conjugate of NBP2-27149. An intracellular stain was performed on Jurkat cells with TLR4 antibody (76B357.1) NBP2-27149PE (blue) and an isotype control MAB004 (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 phycoerythrin.

Immunocytochemistry/Immunofluorescence: TLR4 Antibody (76B357.1) [NB100-56566] - RH-30 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-TLR4 Antibody (76B357.1) NB100-56566 at 1 ug/ml overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.

Immunohistochemistry: TLR4 Antibody (76B357.1) [NB100-56566] - Immunofluorescent staining of TMAs. Representative tissue cores from normal (I), adenomatous polyps (II), and CRC (III and IV) are shown. Image collected and cropped by CiteAb from the following publication (http://www.jeccr.com/content/33/1/45), licensed under a CC-BY license.
Immunohistochemistry-Paraffin: TLR4 Antibody (76B357.1) [NB100-56566] - Analysis of rat salivary gland tissue section at 1:100 dilution. The antibody generated a membrane-cytoplasmic staining in the tissue with stronger signal in ductal epithelial cells.

Immunohistochemistry-Paraffin: TLR4 Antibody (76B357.1) [NB100-56566] - Tissue section of normal human skin stained with antibody at 5 ug/mL. Membrane-cytoplasmic immunopositivity of TLR4 was primarily observed in the pigmented basal cells and the adjacent keratinocytes in the epidermal layer.

Flow Cytometry: TLR4 Antibody (76B357.1) [NB100-56566] - An intracellular stain was performed on RH30 cells with TLR4 Antibody (76B357.1) NB100-56566 (blue) and a matched mouse IgG2b Kappa isotype control (orange) MAB004. 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, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (35503, Thermo Fisher).

Immunohistochemistry-Paraffin: TLR4 Antibody (76B357.1) [NB100-56566] - Human skin stained with at 5 ug/mL, peroxidase-conjugate and DAB chromogen. Staining of formalin-fixed tissues is enhanced by boiling tissue sections in 10 mM sodium citrate buffer, pH 6.0 for 10-20 min followed by cooling at RT for 20 min.
Immunohistochemistry-Paraffin: TLR4 Antibody (76B357.1) [NB100-56566] - Analysis of TLR4 in FFPE human colon tissue using an isotype control (top) and NB100-56566 (bottom) at 5 ug/mL.

Immunohistochemistry-Paraffin: TLR4 Antibody (76B357.1) [NB100-56566] - Human testis tissue stained with antibody at 5 ug/mL.

Immunohistochemistry-Frozen: TLR4 Antibody (76B357.1) [NB100-56566] - This image is TLR4(green) and nucleus(blue) at area postrema of the adult male mouse brain, x20 magnification. Primary antibody diluted 1:500. IHC-Fr image submitted by a verified customer review.

Flow Cytometry: TLR4 Antibody (76B357.1) [NB100-56566] - Analysis of formaldehyde fixed THP-1 cells (human monocytic leukemia cells) using 2 ug/10^6 cells TLR4 antibody (clone 76B357.1) with detection employing a donkey anti-mouse IgG (H+L) cross adsorbed secondary antibody, (DyLight 488 conjugated). Isotype control samples incubated with mouse IgG2b isotype control antibody were processed in parallel under the same assay conditions.
Flow Cytometry: TLR4 Antibody (76B357.1) [NB100-56566] - An intracellular stain was performed on Jurkat cells with TLR4 Antibody (76B357.1) NB100-56566 (blue) and a matched mouse IgG2b Kappa isotype control (orange) MAB004. 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, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (35503, Thermo Fisher).

Flow Cytometry: TLR4 Antibody (76B357.1) [NB100-56566] - An intracellular stain was performed on Raw264.7 cells with TLR4 Antibody (76B357.1) NB100-56566 (blue) and a matched mouse IgG2b Kappa isotype control (orange) MAB004. 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, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (35503, Thermo Fisher).
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<td>Glia-Selective Deletion of Complement C1q Prevents Radiation-Induced Cognitive Deficits and Neuroinflammation</td>
<td>Cancer Research</td>
<td>2021-04-01</td>
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<td>Acute inflammation down-regulates alpha-synuclein expression in enteric neurons</td>
<td>Journal of Neurochemistry</td>
<td>2019-03-01</td>
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<td>HIV Tat-Mediated Induction of Monocyte Transmigration Across the Blood-Brain Barrier: Role of Chemokine Receptor CXCR3</td>
<td>Frontiers in Cell and Developmental Biology</td>
<td>2021-08-30</td>
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<td>Qingwenzhike Prescription Alleviates Acute Lung Injury Induced by LPS via Inhibiting TLR4/NF-kB Pathway and NLRP3 Inflammasome Activation Frontiers in Pharmacology</td>
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<td>Electroacupuncture at Fengchi(GB20) and Yanglingquan(GB34) Ameliorates Paralgesia through Microglia-Mediated Neuroinflammation in a Rat Model of Migraine Brain Sciences</td>
<td>Brain Sciences</td>
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<td>The S1P receptor 1 antagonist Ponesimod reduces TLR4-induced neuroinflammation and increases A? clearance in 5XFAD mice</td>
<td>EBioMedicine</td>
<td>2023-07-20</td>
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<td>Cisplatin cycles treatment sustains cardiovascular and renal damage involving TLR4 and NLRP3 pathways</td>
<td>Pharmacology research &amp; perspectives</td>
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<td>Arjunolic acid modulate pancreatic dysfunction by ameliorating pattern recognition receptor and canonical Wnt pathway activation in type 2 diabetic rats</td>
<td>Life sciences</td>
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<td>(Simple Western, IHC, Rat)</td>
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<td>Contribution of Elevated Glucose and Oxidized LDL to Macrophage Inflammation: A Role for PRAS40/Akt-Dependent Shedding of Soluble CD14 Antioxidants</td>
<td>Pharmacology research &amp; perspectives</td>
<td>2023-05-11</td>
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<td>(WB, Human)</td>
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<td>Endothelial Toll-like receptor 4 is required for microglia activation in the murine retina after systemic lipopolysaccharide exposure</td>
<td>Journal of neuroinflammation</td>
<td>2023-02-04</td>
<td>36739425</td>
<td>(FLOW, Mouse)</td>
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<td>Staphylococcus aureus induces mammary gland fibrosis through activating the TLR/NF-kB and TLR/AP-1 signaling pathways in mice</td>
<td>Microb Pathog</td>
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Details:
Citation using the Azide Free version of this antibody.

More publications at [http://www.novusbio.com/NB100-56566](http://www.novusbio.com/NB100-56566)
Procedures

Western Blot protocol for TLR4 Antibody (NB100-56566)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute anti-TLR4 primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST 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.
Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.
1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 10^5 and 1 x 10^6 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.
   a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.
4. Re-suspend cells to a concentration of 1 x 10^6 cells/mL in staining buffer (NBP2-22647).
5. Aliquot out 100 uL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.
Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:
1. Fix the cells by adding 100 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabilization buffer to every 1 x 10^6 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.
   a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.
   b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).
3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.
Immunohistochemistry-Paraffin Protocol for TLR4 Antibody (NB100-56566)
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 (keep slides in the sodium citrate buffer all the time).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.

Immunocytochemistry/Immunofluorescence Protocol for TLR4 Antibody (NB100-56566)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeabilization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPI if required.
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 100% guarantee, please visit www.novusbio.com/guarantee

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Products Related to NB100-56566

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