**Product Datasheet**

**TLR4 Antibody (76B357.1)**

**NB100-56566**

Unit Size: 0.1 mg

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

Reviews: 5  Publications: 94

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Updated 9/7/2020 v.20.1

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## Product Information

<table>
<thead>
<tr>
<th><strong>Unit Size</strong></th>
<th>0.1 mg</th>
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<tbody>
<tr>
<td><strong>Concentration</strong></td>
<td>1.0 mg/ml</td>
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<tr>
<td><strong>Storage</strong></td>
<td>Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td><strong>Clonality</strong></td>
<td>Monoclonal</td>
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<tr>
<td><strong>Clone</strong></td>
<td>76B357.1</td>
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<tr>
<td><strong>Preservative</strong></td>
<td>0.05% Sodium Azide</td>
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<tr>
<td><strong>Isotype</strong></td>
<td>IgG2b Kappa</td>
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<tr>
<td><strong>Purity</strong></td>
<td>Protein G purified</td>
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<tr>
<td><strong>Buffer</strong></td>
<td>PBS</td>
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</tbody>
</table>

## Product Description

**Host** Mouse

**Gene ID** 7099

**Gene Symbol** TLR4

**Species** Human, Mouse, Rat, Porcine, Bovine, Mammal

**Reactivity Notes** Ground squirrel reactivity reported by a customer review. Mammal reactivity reported in scientific literature (PMID: 25130694). Porcine reactivity reported in scientific literature (PMID: 29941006). Bovine reactivity reported in scientific literature (PMID: 19528595). Sheep (85%), Cat, Equine (78%). Mammal reactivity reported in scientific literature (PMID: 25130694).

**Immunogen** This antibody was developed against a portion of amino acids 100-200 of human TLR4 (NP_612564).

## Product Application Details

**Applications** Western Blot, Chromatin Immunoprecipitation, Dot Blot, ELISA, Flow Cytometry, Flow (Cell Surface), Flow (Intracellular), Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, In vitro assay, Block/Neutralize, CyTOF-ready, Dual RNAscope ISH-IHC, ELISA Capture (Matched Antibody Pair), Knockdown Validated, Knockout Validated

**Recommended Dilutions** Western Blot 1-3 ug/ml, Chromatin Immunoprecipitation 1:10-1:500, 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, Flow (Cell Surface), Flow (Intracellular), ELISA Capture (Matched Antibody Pair) 0.5 ug, CyTOF-ready, Knockout Validated, Knockdown Validated, Block/Neutralize, Dual RNAscope ISH-IHC

**Application Notes** Flow (Intracellular) use reported in literature (See Cohen et al, 2008); Immunofluorescence (See Nowicki et al, 2009); IHC-F (Nowicki et al, 2012); Flow (cell surface) use reported in scientific literature (PMID: 23796194). Use in chromatin immunoprecipitation reported in scientific literature (PMID: 21966468). Use In vitro reported in scientific literature (PMID: 26446256). Use in blocking/neutralizing reported in scientific literature (PMID 26063617). Use in ELISA reported in scientific literature (PMID: 30139760). Use in Dot blot reported in scientific literature (PMID: 27248820). Use in Knockout Validated reported in scientific literature (PMID: 31125703). Knockdown validation (PMID: 31766635). This TLR4 antibody is CyTOF ready.
Dual RNAscope ISH-IHC: TLR4 Antibody (76B357.1) [NB100-56566] - Formalin-fixed paraffin-embedded tissue sections of human tonsil were probed for TLR4 mRNA (ACD RNAscope Probe, catalog #311281; Fast Red chromogen, ACD catalog # 322750). Adjacent tissue section was processed for immunohistochemistry using Mouse Monoclonal (Novus Biologicals catalog #NB100-56566) at 5µg/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.

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 licence.

Western Blot: TLR4 Antibody (76B357.1) [NB100-56566] - Analysis using 2 µg/ml on (A) human intestine and 6 µg/ml on (B) mouse intestine and C) rat intestine lysate.

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 this antibody and 1 µg 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. 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 licence.

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 permeablized 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.

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 licence.

Immunohistochemistry-Paraffin: TLR4 Antibody (76B357.1) [NB100-56566] - Analysis of Rat's 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 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] - 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 parallal under the same assay conditions.

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 paraffin-embedded formalin-fixed 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 using 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. This image was submitted via customer Review.
<table>
<thead>
<tr>
<th>Publications</th>
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<tbody>
<tr>
<td>Seimetz M, Sommer N, Bednorz M et al. NADPH oxidase subunit NOXO1 is a target for emphysema treatment in COPD Nat Metab Jun 1 2020 12:00AM [PMID: 32694733]</td>
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<tr>
<td>Ozbek M, Hitit M, Ergun E et al. Expression profile of Toll-like receptor 4 in rat testis and epididymis throughout postnatal development Andrologia Jan 31 2020 12:00AM [PMID: 32003057] (IHC, Rat)</td>
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<tr>
<td>Lacave-Lapalun JV, Benderitter M, Linard C Flagellin and LPS each restores rat lymphocyte populations after colorectal irradiation J Leukoc Biol. 2014 Jun [PMID: 24532644] (Flow, Rat)</td>
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<td>Details: Citation using the Azide Free format of this antibody.</td>
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<td>Rodet F, Capuz A, Ozcan BA et al. PC1/3 KD Macrophages Exhibit Resistance to the Inhibitory Effect of IL-10 and a Higher TLR4 Activation Rate, Leading to an Anti-Tumoral Phenotype Cells Nov 22 2019 12:00AM [PMID: 31766635] (ICC/IF, KD, Rat)</td>
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More publications at [http://www.novusbio.com/NB100-56566](http://www.novusbio.com/NB100-56566)
Western Blot protocol for TLR4 Antibody (NB100-56566)
TLR4 Antibody (76B357.1): https://www.novusbio.com/products/tlr4-antibody-76b3571_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.
Flow (Intracellular) protocol for TLR4 Antibody (NB100-56566)

TLR4 Antibody (76B357.1): https://www.novusbio.com/products/trl4-antibody-76b3571_nb100-56566

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 (NBP2-26247).
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.

www.novusbio.com  technical@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 100% guarantee, please visit www.novusbio.com/guarantee

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