

# Product Datasheet

## TLR2 Antibody - BSA Free NB100-56720

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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**NB100-56720**

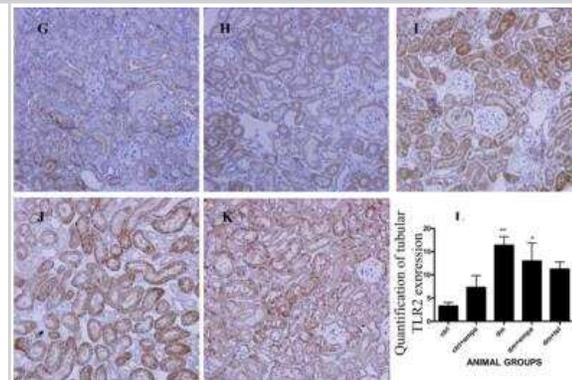
TLR2 Antibody - BSA Free

<b>Product Information</b>	
<b>Unit Size</b>	0.1 mg
<b>Concentration</b>	1.0 mg/ml
<b>Storage</b>	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
<b>Clonality</b>	Polyclonal
<b>Preservative</b>	0.05% Sodium Azide
<b>Isotype</b>	IgG
<b>Purity</b>	Protein G purified
<b>Buffer</b>	PBS
<b>Target Molecular Weight</b>	89 kDa
<b>Product Description</b>	
<b>Description</b>	Novus Biologicals Rabbit TLR2 Antibody - BSA Free (NB100-56720) is a polyclonal antibody validated for use in IHC, WB, Flow, ICC/IF and IP. Anti-TLR2 Antibody: Cited in 20 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
<b>Host</b>	Rabbit
<b>Gene ID</b>	7097
<b>Gene Symbol</b>	TLR2
<b>Species</b>	Human, Mouse
<b>Immunogen</b>	This antibody was developed against a mixture of two synthetic peptides found between amino acids 150-200 and 300-400 of human TLR2 (NP_003255).
<b>Product Application Details</b>	
<b>Applications</b>	Western Blot, Immunohistochemistry-Paraffin, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunoprecipitation
<b>Recommended Dilutions</b>	Western Blot 1-3 ug/ml, Flow Cytometry reported in scientific literature (PMID 31285077), Immunohistochemistry 1:10-1:500, Immunocytochemistry/ Immunofluorescence reported in scientific literature (PMID 30618413), Immunoprecipitation reported in scientific literature (PMID 31383741), Immunohistochemistry-Paraffin 1:10-1:500
<b>Application Notes</b>	In transfected human TLR2 cell lysate, a 90 kDa band is observed. This has also been reported to work well in bronchial epithelial cells (UBH16E) and THP-1 cells.

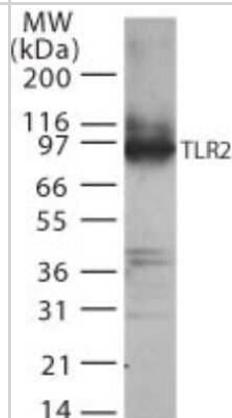


## Images

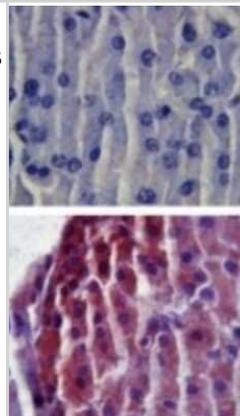
TLR2-Antibody-Immunohistochemistry-NB100-56720-img0006.jpg



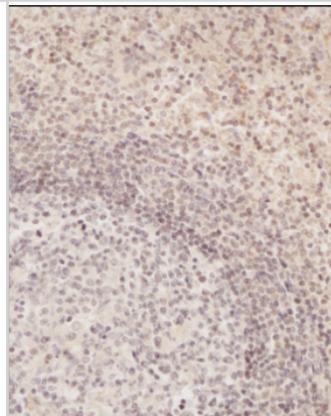
Western Blot: TLR2 Antibody [NB100-56720] - Analysis using the Azide Free version of NB100-56720. Detection of TLR2 in transfected cell lysate using this antibody.



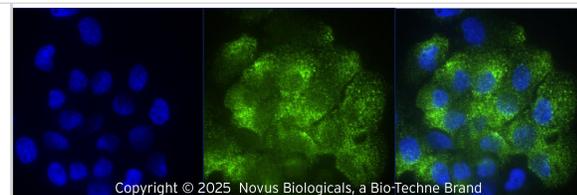
Immunohistochemistry-Paraffin: TLR2 Antibody [NB100-56720] - Analysis of mouse pancreas tissue using an isotype control (top) and this antibody (bottom) at 5 ug/ml.



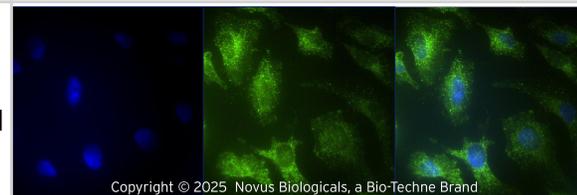
Analysis of a FFPE tissue section of human spleen using 1:200 dilution of TLR2 antibody. The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.



TLR2 was detected in immersion fixed A431 human skin carcinoma cell line using Rabbit anti-TLR2 Protein G purified Polyclonal Antibody (Catalog # NB100-56720) at 1.0 µg/mL overnight at 4C. Cells were stained using DyLight 488-conjugated Anti-Rabbit IgG (H+L) Cross-Absorbed Secondary Antibody (green), and counterstained with DAPI (blue). Cells were imaged using a 40X objective.



TLR2 was detected in immersion fixed HeLa human cervix adenocarcinoma cell line using Rabbit anti-TLR2 Protein G purified Polyclonal Antibody (Catalog # NB100-56720) at 1.0 µg/mL overnight at 4C. Cells were stained using DyLight 488-conjugated Anti-Rabbit IgG (H+L) Cross-Absorbed Secondary Antibody (green), and counterstained with DAPI (blue). Cells were imaged using a 40X objective.



## Publications

Doğan G, Sandıkçı M, Karageç L. et Al. Stage-specific expression of Toll-like receptors in the seminiferous epithelium of mouse testis *Histochem Cell Biol* 2024-07-31 [PMID: 39085445]

Neuper T, Frauenlob T, Dang HH et al. ADP-heptose attenuates *Helicobacter pylori* -induced dendritic cell activation *Gut Microbes* 2024-09-17 [PMID: 39288239]

Doğan G, Karageç N, Esmen K et al. Expression of Toll-Like Receptors in the Lung Tissue of Mouse Fetuses Generated by in vitro Embryo Culture and Embryo Transfer Cells, tissues, organs 2023-04-27 [PMID: 37105136] (WB, Mouse)

Millar FR, Pennycuik A, Muir M Et al. Toll-like receptor 2 orchestrates a tumor suppressor response in non-small cell lung cancer *Cell Rep* 2022-11-09 [PMID: 36351380] (IHC-P, Human, Mouse)

### Details:

Citation using the Azide Free version of this antibody.

Yoshimoto T, Kittaka M, Doan AAP et al. Osteocytes directly regulate osteolysis via MYD88 signaling in bacterial bone infection *Nature communications* 2022-11-04 [PMID: 36333322] (IHC-P, Mouse)

Khandelwal N, Shaikh M, Mhetre A et al. Fatty acid chain length drives lysophosphatidylserine-dependent immunological outputs *Cell chemical biology* 2021-01-21 [PMID: 33571455]

Eyking, A;Ferber, F;Köhler, S;Reis, H;Cario, E; TRIM58 Restrains Intestinal Mucosal Inflammation by Negatively Regulating TLR2 in Myeloid Cells *J. Immunol.* 2019-08-05 [PMID: 31383741] (WB, IP, Human)

Arya RP, Arankalle VA Phenotypic analysis of monocytes and CD4+ T cells in hepatitis E patients with or without pregnancy *Hum. Immunol.* 2019-07-05 [PMID: 31285077] (FLOW, Human)

Fan Y, Yang L, Wei Q et al. Toll-like receptor 10 (TLR10) exhibits suppressive effects on inflammation of prostate epithelial cells. *Asian J. Androl.* 2019-01-01 [PMID: 30618413] (ICC/IF, IHC-P, Human)

Zou Weifeng, He Fang, Liu Sha et al. PM2.5 Induced the Expression of Fibrogenic Mediators via HMGB1-RAGE Signaling in Human Airway Epithelial Cells. *Canadian Respiratory Journal* 2018-01-01 [PMID: 29670673] (WB, Human)

Sumedha S, Kotrashetti VS, Nayak RS, Nayak A et al. Immunohistochemical localization of TLR2 and CD14 in gingival tissue of healthy individuals and patients with chronic periodontitis *Biotech Histochem* 2017-09-14 [PMID: 28910171] (Human)

Krishnan S, Chen S, Turcatel G et al. Regulation of Toll-like receptor 2 interaction with Ecgp96 controls *Escherichia coli* K1 invasion of brain endothelial cells *Cell Microbiol.* 2013-01-01 [PMID: 22963587] (WB, Human)

More publications at <http://www.novusbio.com/NB100-56720>



## Procedures

### Western Blot Protocol for TLR2 Antibody (NB100-56720)

#### 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 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 manufacturer's instructions.

### Immunocytochemistry/ Immunofluorescence Protocol for TLR2 Antibody (NB100-56720)

#### 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 4% paraformaldehyde to the dish and fix at room temperature for 10 minutes.
2. Remove the paraformaldehyde and wash the cells in PBS.
3. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 2 min.
4. Remove the permeabilization buffer and wash three times for 5 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 5 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 5 minutes each.
10. Counter stain DNA with DAPI if required.



**Immunohistochemistry-Paraffin Protocol for TLR2 Antibody (NB100-56720)**

## 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 at all times).

## 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.
13. Mount coverslips.



## Flow (Cell Surface) Protocol for TLR2 Antibody (NB100-56720)

### Protocol for Flow Cytometry Cell Surface Staining

#### Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between  $2 \times 10^5$  and  $1 \times 10^6$  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  $\mu$ L for counting, then transfer cell volume into a 15 mL conical tube and centrifuge for 4 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  $1 \times 10^6$  cells/mL in staining buffer.
5. Aliquot out 100  $\mu$ L 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.

#### Cell surface staining

1. Recommended: Block non-specific interactions using 0.5-1  $\mu$ g of a species specific Fc-blocking reagent.
2. Add appropriate amount of each antibody (eg. 1 test or 1  $\mu$ g per sample, as experimentally determined) to 100  $\mu$ L of staining buffer per sample (eg. use 1 mL of staining buffer for 10 samples).
3. Mix well and incubate at room temperature in dark for 20 minutes.
4. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
5. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200  $\mu$ L for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
6. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
7. Incubate at room temperature in dark for 20 minutes.
8. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200  $\mu$ L for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Resuspend in an appropriate volume of staining buffer (usually 500  $\mu$ L per sample) and proceed with analysis on your flow cytometer.



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## Products Related to NB100-56720

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NBL1-16952	TLR2 Overexpression Lysate
NB100-56720PEP	TLR2 Antibody Blocking Peptide
NBP2-25297	Pam3CSK4, TLR1 and TLR2 Ligand
NBP2-29331	TIRAP (TLR2 and 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

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### 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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