# **Product Datasheet**

# TLR9 Antibody (26C593.2) - BSA Free NBP2-24729

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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#### NBP2-24729

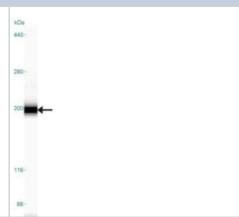
TLR9 Antibody (26C593.2) - BSA Free

TLR9 Antibody (26C593.2) - BSA Free	
Product Information	
Unit Size	0.1 mg
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	26C593.2
Preservative	0.05% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS
Product Description	
Host	Mouse
Gene ID	54106
Gene Symbol	TLR9
Species	Human, Mouse, Rat, Canine, Equine, Primate, Monkey
Reactivity Notes	Rhesus Monkey.
Immunogen	This antibody was developed against KLH-conjugated synthetic peptide corresponding to amino acids 268-300 of human TLR9 isoform A (Genbank accession no. AAF78037).
Product Application Details	
Applications	Western Blot, Simple Western, Dot Blot, ELISA, Flow Cytometry, Flow (Intracellular), Functional, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, In vitro assay, Immunoprecipitation, Block/Neutralize, CyTOF-ready, Knockdown Validated
Recommended Dilutions	Western Blot 2-5 ug/ml, Simple Western 30 ug/ml, Flow Cytometry, ELISA 1:100 - 1:2000. Use reported in multiple pieces of scientific literature, Immunohistochemistry reported in scientific literature (PMID 27744078), Immunocytochemistry/ Immunofluorescence 1:10-1:500, Immunoprecipitation 1:10 - 1:500. Use reported in scientific literature (PMID 25871979), Immunohistochemistry-Paraffin 5 ug/ml, Functional reported in scientific literature (PMID 25411258), In vitro assay reported in scientific literature (PMID 27248820), Flow (Intracellular) 1:10 - 1:1000. Use reported in scientific literature (PMID 24986635), CyTOF-ready, Knockdown Validated reported in scientific literature (PMID 31655343), Block/Neutralize reported in scientific literature (PMID 25338738)
Application Notes	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. In human PBMC, a ~120 kDa band is observed. A smaller isoform, TLR9 isoform B (Genbank accession no. AAF72190) containing 975 amino acids may also be observed in some cases. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue.





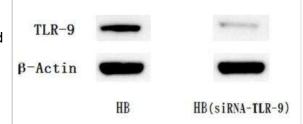
Simple Western: TLR9 Antibody (26C593.2) [NBP2-24729] - Lane view shows a specific band for TLR9 in 0.5 mg/ml of Ramos lysate. This experiment was performed under reducing conditions using the 66-440 kDa separation system. Image using the Azide Free format of this antibody.



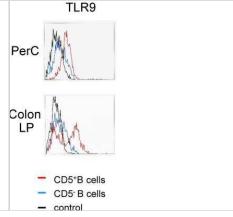
Flow Cytometry: TLR9 Antibody (26C593.2) [NBP2-24729] - Expression of TLR9 protein on epithelial cells. HNEC, Detroit-562 and FaDu were stained intracellularly with PE-Ab against TLR9 (open histograms) or appropriate isotype control (shaded histograms) and analyzed by flow cytometry. Representative pictures from one out of three independent experiments are shown. Image collected and cropped by CiteAb from the following publication (//dx.plos.org/10.1371/journal.pone.0098239), licensed under a CC-BY license.



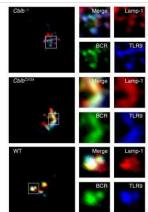
Knockdown Validated: TLR9 Antibody (26C593.2) [NBP2-24729] - Expression of TLR-9 protein in HB cells before and after 48 hr of transfection. Beta-actin was used as an internal control. Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0092748) licensed under a CC-BY license.



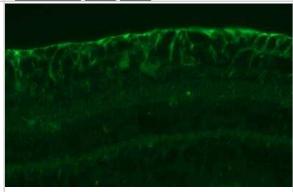
Flow Cytometry: TLR9 Antibody (26C593.2) [NBP2-24729] - Analysis of colonic LP or peritoneal cavity (PerC) of normal mice were evaluated by flow cytometry. B cells were intracellularly stained with the anti-TLR9 antibody after the cell surface staining with anti-B220 and CD5 antibodies, and examined using flow cytometry. N = 3, performed twice. Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0146191) licensed under a CC-BY license.



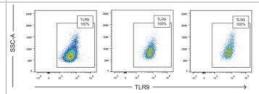
Immunocytochemistry/Immunofluorescence: TLR9 Antibody (26C593.2) [NBP2-24729] - Cbl-b is required for the endocytic transit of TLR9. Representative confocal microscopic images of splenocytes from mice with indicated genotypes. For experiments, cells were stimulated through the BCR (green) for 30 minutes then fixed and stained for TLR9 (blue) and Lamp-1 (red)(n = 3). Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0089792) licensed under a CC-BY license.



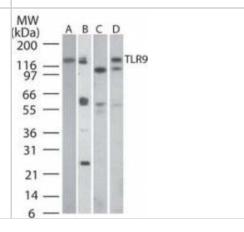
Immunohistochemistry-Paraffin: TLR9 Antibody (26C593.2) [NBP2-24729] - Monkey retina tissue. Image from verified customer review.



Flow Cytometry: TLR9 Antibody (26C593.2) [NBP2-24729] - Gating strategy for the detection of TLR-7 and -9 in B cell subsets. PBMC were isolated from whole blood and stained for surface markers before cells were fixed, permeabilised and stained for TLR-9. FSC and SSC were first used to gate out debris and SSC-A and SSC-H was utilized to eliminate duplicates. Further gating was done on CD45 and CD19, to target B cells. To separate between the different B cell populations, we gated on CD27 and IgD (D), followed by TLR-9 expression on these subsets. Data from one representative patient is shown. Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0120383) licensed under a CC-BY license.



Western Blot: TLR9 Antibody (26C593.2) [NBP2-24729] - Analysis of TLR9 in A) human PBMC, B) human intestine, C) mouse intestine, and D) rat intestine tissue lysates using this antibody at a dilution of 3 ug/ml.



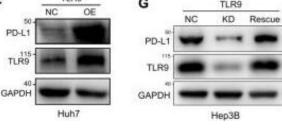
Flow Cytometry: TLR9 Antibody (26C593.2) [NBP2-24729] - Expression of TLR9 on turbinate epithelial cells from healthy controls compared to turbinate and polyp epithelial cells from patients with CRSwNP, n = 5 (A). Intracellular staining for TLR9 (open histogram, black line) and isotype control (filled histogram) on turbinate epithelial cells from a healthy control (B), turbinate epithelial cells (C) and polyp epithelial cells from a patient (D), analysed using flow cytometry. Results are presented as mean +/- SEM, \*\*P<0.01. Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0105618) licensed under a CC-BY license.

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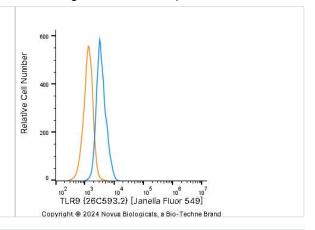
1. System of the state o

TLR9 activation upregulated PD-L1 expression by promoting STAT3 Tyr705 phosphorylation in HCC cells. (A) PD-L1 protein expression after treatment with ODN2216 with different concentrations or the indicated times (different concentrations: 0, 5, 10, or 20uM; times: 0, 6, 12, 24, and 36 hours in 10 uM) in Hep3B cells. PD-L1 protein levels were analyzed by Western blotting. (B) PD-L1 protein expression after treatment with ODN2216 with different concentrations or the indicated times (different concentrations: 0, 2.5, 5, or 10uM; times: 0, 6, 12, 24, and 36 hours in 10 uM) in Huh7 cells. PD-L1 protein levels were analyzed by Western blotting. (C) PD-L1 protein expression after treatment with ODN1585 with different concentrations or the indicated times (different concentrations: 0, 2.5, 5, and 10 uM; times: 0, 6, 12, 24, and 36 hours in 5uM) in Hepa1-6 cells. PD-L1 protein levels were analyzed by Western blotting. (D) PD-L1+ tumor cells were detected by flow cytometry after TLR9 agonist (Hep3B and Huh7 cells with ODN2216; Hepa1-6 cells with ODN1585) treatment with indicated concentration. (values are mean +/- SD, \*p < 0.05, \*\*p < 0.01, \*\*\*p<0.001, NS indicates no significance). (E) PD-L1 expression after TLR9 overexpression in Huh7 cells. PD-L1 expression levels were analyzed by immunofluorescence. (F) PD-L1 protein expression after TLR9 overexpression in Huh7 cells. PD-L1 protein levels were analyzed by Western blotting. (G) PD-L1 protein expression in TLR9 knockdown or TLR9 rescue Hep3B cells. PD-L1 protein levels were analyzed by Western blotting. (H and I) mRNA levels of PD-L1 in Hep3B (H) and Huh7 (I) cells measured by qRT-PCR after stimulation with different concentrations of ODN2216. (values are mean +/- SD, \*p < 0.05, \*\*p < 0.01, NS indicates no significance) (J) TLR9 overexpressioninduced PD-L1 expression after MYC, JUN, IRF1, IRF3, STAT1 or STAT3 silencing. PD-L1 mRNA expression in Huh7 cells was analyzed after TLR9 overexpression alone or in the presence of MYC-, JUN-, IRF1 -, IRF3-, STAT1- or STAT3-specific siRNA or siRNA-NC. (values are mean +/- SD, \*\*\*p<0.001). (K) p-STAT3 (Tyr705) levels in Hep3B cells after treatment with different concentrations of ODN2216 (a TLR9 agonist; ODN2216: 0, 2.5, 5, or 10uM) analyzed by Western blotting. (L) p-STAT3 (Tyr705) levels after TLR9 overexpression in Huh7 cells analyzed by Western blotting. (M) TLR9 overexpression-induced p-STAT3 (Tyr705) levels after TLR9 inhibition. p-STAT3 (Tyr705) levels were analyzed after TLR9 overexpression alone or in the presence of the TLR9 antagonist chloroquine diphosphate. (N) p-STAT3-induced PD-L1 levels after STAT3 inhibition. PD-L1 levels were analyzed after TLR9 overexpression alone or in the presence of the STAT3-specific small molecular inhibitor BP-1-102. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32483468), licensed under a CC-BY



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THP-1 human acute monocytic leukemia cell line was stained with Mouse anti-TLR9 (26C593.2) Protein-G purified Monoclonal Antibody conjugated to Janelia Fluor® 549 (Catalog # NBP2-24729JF549, blue histogram) or matched control antibody (orange histogram).



#### **Publications**

Yu JI, Kim JH, Nam KE et al. Pneumococcal ?pep27 Immunization Attenuates TLRs and NLRP3 Expression and Relieves Murine Ovalbumin-Induced Allergic Rhinitis Journal of Microbiology and Biotechnology 2022-06-28 [PMID: 35484967] (WB)

Spurgeon BEJ, Frelinger AL Platelet Phenotyping by Full Spectrum Flow Cytometry Current protocols 2023-02-01 [PMID: 36779850]

Ma X, Rawnsley D, Kovacs A et al. TRAF2, an Innate Immune Sensor, Reciprocally Regulates Mitophagy and Inflammation to Maintain Cardiac Myocyte Homeostasis JACC Basic Transl Sci 2022-04-12 [PMID: 35411325]

Lam LKM, Dobkin J, Eckart KA et al. Bat Red Blood Cells express Nucleic Acid Sensing Receptors and bind RNA and DNA Immunohorizons 2022-05-20 [PMID: 35595326]

Li Z, Fu WJ, Chen XQ et al. Autophagy-based unconventional secretion of HMGB1 in glioblastoma promotes chemosensitivity to temozolomide through macrophage M1-like polarization Journal of experimental & clinical cancer research: CR 2022-02-22 [PMID: 35193644] (ICC/IF, Human)

Khin PP, Hong Y, Yeon M Et al. Dulaglutide improves muscle function by attenuating inflammation through OPA-1-TLR-9 signaling in aged mice Aging 2021-09-19 [PMID: 34537761] (WB, Mouse)

Ishizu T, Eichin D, Padzik A et al. Head and neck squamous cell carcinoma cell lines have an immunomodulatory effect on macrophages independent of hypoxia and toll-like receptor 9 BMC cancer 2021-09-03 [PMID: 34479492] (WB, Human)

Branchi V, Esser L, Boden C et al. A Combined TLR7/TLR9/GATA3 Score Can Predict Prognosis in Biliary Tract Cancer Diagnostics (Basel, Switzerland) 2021-09-01 [PMID: 34573939] (IF/IHC, Human)

Kuramoto K, Kim YJ, Hong JH, He C The autophagy protein Becn1 improves insulin sensitivity by promoting adiponectin secretion via exocyst binding Cell reports 2021-05-25 [PMID: 34038729]

Li FJ, Surolia R, Li H et al. Citrullinated vimentin mediates development and progression of lung fibrosis Science translational medicine 2021-03-17 [PMID: 33731433]

KorimovA A, DubovY P N-Formylated Peptide Induces Increased Expression of Both Formyl Peptide Receptor 2 (Fpr2) and Toll-Like Receptor 9 (TLR9) in Schwannoma Cells-An In Vitro Model for Early Inflammatory Profiling of Schwann Cells Cells 2020-12-11 [PMID: 33322305] (WB, Rat)

Niemczyk G, Fus L, Czarzasta K et al. Expression of Toll-Like Receptors in the Animal Model of Bladder Outlet Obstruction BioMed research international 2020-12-12 [PMID: 33381567] (IF/IHC, Rat)

More publications at <a href="http://www.novusbio.com/NBP2-24729">http://www.novusbio.com/NBP2-24729</a>





### **Novus Biologicals USA**

10730 E. Briarwood Avenue Centennial, CO 80112

USA

Phone: 303.730.1950 Toll Free: 1.888.506.6887

Fax: 303.730.1966

nb-customerservice@bio-techne.com

#### **Bio-Techne Canada**

21 Canmotor Ave Toronto, ON M8Z 4E6

Canada

Phone: 905.827.6400 Toll Free: 855.668.8722 Fax: 905.827.6402

canada.inquires@bio-techne.com

#### **Bio-Techne Ltd**

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.EMEA@bio-techne.com

#### **General Contact Information**

www.novusbio.com

Technical Support: nb-technical@bio-

techne.com

Orders: nb-customerservice@bio-techne.com

General: novus@novusbio.com

## **Products Related to NBP2-24729**

NBP2-26232 CpG oligodeoxynucleotides with negative control, TLR9 ligand

HAF007 Goat anti-Mouse IgG Secondary Antibody [HRP]

NB720-B Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]

NBP1-43319-0.5mg Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)

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