

# Product Datasheet

## TLR3 Antibody (40C1285.6) - BSA Free NBP2-24875

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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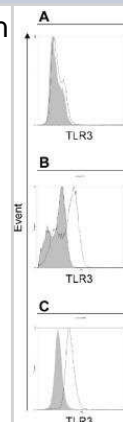
**NBP2-24875**

TLR3 Antibody (40C1285.6) - BSA Free

Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	40C1285.6
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS
Product Description	
Host	Mouse
Gene ID	7098
Gene Symbol	TLR3
Species	Human, Mouse, Canine
Immunogen	A synthetic peptide corresponding to amino acids 55-85 of human TLR3 was used as immunogen.
Product Application Details	
Applications	Western Blot, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Block/Neutralize, Bioactivity
Recommended Dilutions	Western Blot 1-3 ug/ml, Flow Cytometry 2-4 ug/ 1x10 <sup>6</sup> cells, Immunohistochemistry 1:10-1:500, Immunocytochemistry/ Immunofluorescence 1:10-1:500, Immunohistochemistry-Paraffin 1:10-1:500, Flow (Intracellular) reported in scientific literature (PMID 24836676), Bioactivity reported in scientific literature (PMID 28894449), Block/Neutralize reported in scientific literature (PMID 28894449)

**Images**

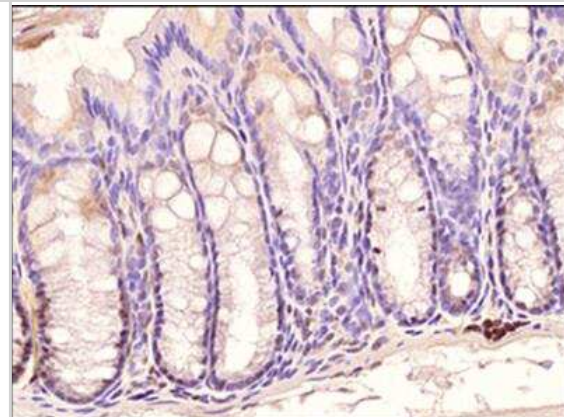
Flow Cytometry: TLR3 Antibody (40C1285.6) [NBP2-24875] - Expression of TLR3 protein on epithelial cells. HNEC (A), Detroit-562 (B) and FaDu (C) were stained intracellularly with FITC-Abs against TLR3 (open histograms) or appropriate isotype control (shaded histograms) and analyzed by flow cytometry. 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](https://dx.plos.org/10.1371/journal.pone.0098239)), licensed under a CC-BY license.



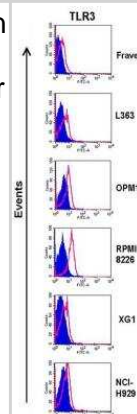
**Western Blot: TLR3 Antibody (40C1285.6) [NBP2-24875]** - All the cell lines displayed a strong expression of TLR3 protein. Cell lysates were electrophoresed and blotted to PVDF membrane, which was probed with TLR3-specific antibody. To confirm the immunoreactivity of the antibody, different positive controls were included. Beta-actin was served as loading control and was used to normalize expression levels between cells. Data are representative for analysis of  $\geq 2$  independent experiments. Image collected and cropped by CiteAb from the following publication ([//doi.org/10.1371/journal.pone.0060671](https://doi.org/10.1371/journal.pone.0060671)) licensed under a CC-BY license.



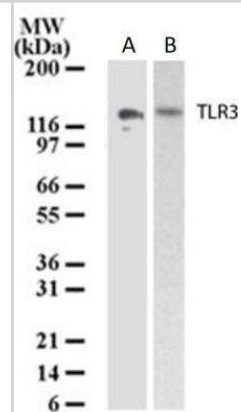
**Immunohistochemistry-Paraffin: TLR3 Antibody (40C1285.6) [NBP2-24875]** - Mouse colon using TLR3 antibody (clone 40C1285.6) at 1:500 dilution with HRP-DAB detection and hematoxylin counterstaining. Intense signal was found in subset of cells at the bases of the crypts.



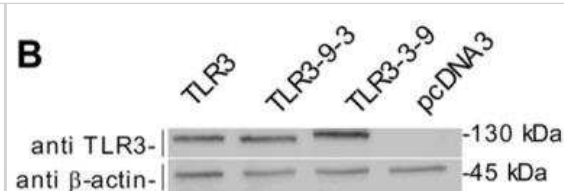
**Flow Cytometry: TLR3 Antibody (40C1285.6) [NBP2-24875]** - Expression of TLR3 in Fravel, L363, OPM1, RPMI8226, XG1, and NCI-H929 as determined by flow cytometry. HCMLs were stained using an intracellular staining protocol with TLR3 antibodies followed by relevant secondary fluorescent-conjugated antibodies. Filled histograms (purple) represent the isotype controls and the open histograms (red) indicate TLR3. Data are representative for analysis of  $\geq 2$  independent experiments. Image collected and cropped by CiteAb from the following publication ([//doi.org/10.1371/journal.pone.0060671](https://doi.org/10.1371/journal.pone.0060671)) licensed under a CC-BY license.



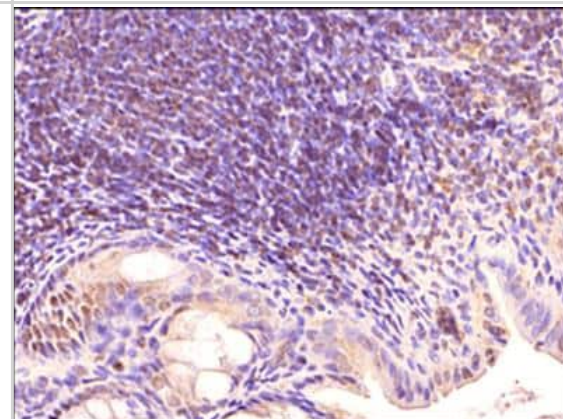
**Western Blot: TLR3 Antibody (40C1285.6) [NBP2-24875]** - Analysis of TLR3 in lysates from A) human intestine and B) ovary using TLR3 antibody at 3 ug/ml. Goat anti-mouse HRP conjugate was used as secondary.



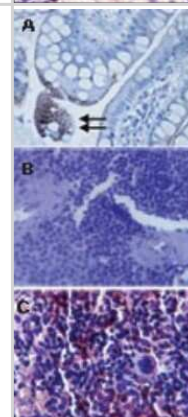
Western Blot: TLR3 Antibody (40C1285.6) [NBP2-24875] - HEK293T cells were transfected with TLR3, TLR3-9-3 and TLR3-3-9. Western blot was performed using anti-TLR3 or anti-HA antibodies. Anti-Beta-actin antibodies were used as a loading control. The representative data from three experiments are shown. Image collected and cropped by CiteAb from the following publication ([//doi.org/10.1371/journal.pone.0092391](https://doi.org/10.1371/journal.pone.0092391)) licensed under a CC-BY license.



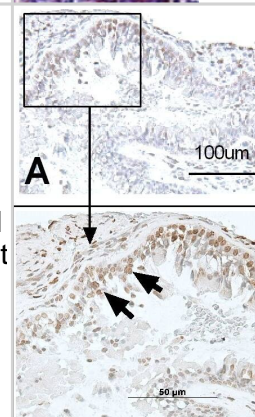
Immunohistochemistry-Paraffin: TLR3 Antibody (40C1285.6) [NBP2-24875] - Tissue section of mouse intestine using TLR3 antibody (clone 40C1285.6) at 1:500 dilution with HRP-DAB detection and hematoxylin counterstaining. The representative image shows a punctate staining of the ER and endosomes in a subset of cells in Peyer's patches (organized lymphoid nodules) in the tested section.



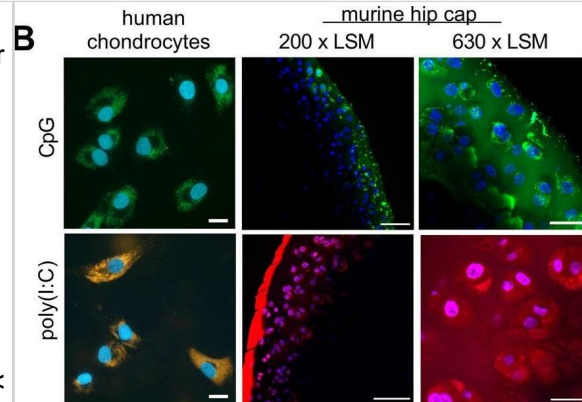
Immunohistochemistry-Paraffin: TLR3 Antibody (40C1285.6) [NBP2-24875] - Analysis of TLR3 in (A) human gut lumen (longitudinal section, transverse region) using this antibody at 10  $\mu$ g/ml (Data courtesy Dr. Elizabeth Furrie, University of Dundee) and mouse spleen tissue using isotype control (B) and this antibody (C) at 5 mg/ml.



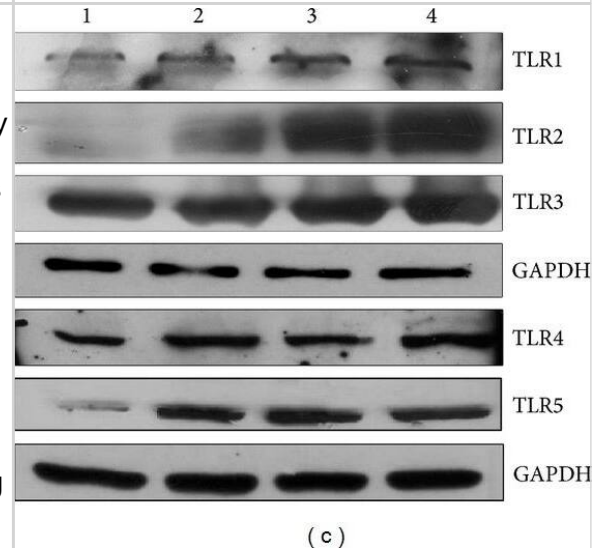
Immunohistochemistry: TLR3 Antibody (40C1285.6) - BSA Free [NBP2-24875] - The nasal epithelium expresses TLR3, TLR7, TLR9, RIG-I & MDA-5. Sections of nasal biopsies were incubated with antibodies against TLR3 (A), TLR7 (B), TLR9 (C), RIG-I (D), & MDA-5 (E) & visualized by 3, 3'-diaminobenzidine (brown). In control slides (F), N-series universal negative control reagent was used. All sections were accompanied with a square magnification. All slides were counterstained with haematoxylin (blue). The figure shows one representative biopsy out of four (3 male, 1 female). The arrows indicate positive stained cells. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/24886842>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



**Immunohistochemistry:** TLR3 Antibody (40C1285.6) - BSA Free [NBP2-24875] - TLRs are expressed & functional in chondrocytes. A RT-PCR for TLR3 & 9 expression in primary human & murine chondrocytes. GAPDH served as loading control. B Confocal microscopy of labelled CpG (green) & poly(I:C) (red) uptake by either primary human chondrocytes (scale bar 20  $\mu$ m) or murine hip caps (scale bar 50  $\mu$ m (centre) 20  $\mu$ m (right)). DAPI (blue) was used as counterstaining for nuclei. C IFN $\alpha$  ELISA of murine chondrocytes stimulated with ODN (one-way ANOVA: F (2, 9) = 2.21, p = 0.16, N = 4). D IFN $\alpha$  ELISA of primary human chondrocytes stimulated with ODN & poly(I:C) (one-way ANOVA: F (3, 11) = 0.75, p = 0.55, N = 4). E IFN $\beta$  ELISA of murine chondrocytes stimulated with poly(I:C) or ODN (one-way ANOVA: F (2, 15) = 59.61, p < 0.0001, N = 6). F IFN $\beta$  ELISA of primary human chondrocytes stimulated with poly(I:C) or ODN (one-way ANOVA: F (2, 6) = 95.43, p = <0.0001, N = 3). G Staurosporine was used to induce cell death. The release of RNA from human primary chondrocytes after treatment was measured in the culture supernatant using the RiboGreen assay (RM ANOVA: F (4, 12) = 101.2, p < 0.0001, N = 4). Dunnett's post-hoc test revealed an increase for 200, 500, & 1000 ng/ml staurosporine treatment compared to the untreated control. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/35277480>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



**Western Blot:** TLR3 Antibody (40C1285.6) - BSA Free [NBP2-24875] - TLR mRNA & protein expression in airlifted immortal human corneal (HCET) & conjunctival epithelial cells (IOBA-NHC). (a) Averaged fold increase of TLR mRNA in IOBA-NHC cells. The increase was statistically significant for each TLR. (b) Averaged fold increase of TLR mRNA in HCET cells. The increase was statistically significant for each TLR. Cells were airlifted for 10 days. TLR mRNA level was determined by qPCR & compared to submerged cells. The experiment was repeated 3 times. Y error bars represent the standard deviation of the averaged results. (c) Micrographs of a representative western blot showing TLR protein expression in IOBA-NHC cells. Lane 1: submerged-cultured; lane 2: 3 days after airlifting culture; lane 3: 7 days after airlifting culture; lane 4: 10 days after airlifting culture. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) protein was probed as loading control. (d) Averaged TLR protein increase in IOBA-NHC cells 10 days after airlifting culture compared to submerged-cultured cells. X-ray films from 2 independent western blot experiments were scanned in a densitometer, & the results were averaged. The increase was statistically significant for each TLR protein tested. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/24976686>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Hiltunen N, Kemi N, Väyrynen JP et al. Toll-like receptors 1-9 in small bowel neuroendocrine tumors-Clinical significance and prognosis PLoS One 2024-05-06 [PMID: 38709790]

Liu W, Wang Y, Liu S et al. E3 Ubiquitin Ligase RNF13 Suppresses TLR Lysosomal Degradation by Promoting LAMP-1 Proteasomal Degradation Adv Sci (Weinh) 2024-06-21 [PMID: 39031743]

Alaimo A, Genovesi S, Annesi N et al. Sterile inflammation via TRPM8 RNA-dependent TLR3-NF-kB/IRF3 activation promotes antitumor immunity in prostate cancer EMBO J 2024-02-05 [PMID: 38316991]

Haddaoui HE, van Eijck CW, Doukas M et al. Rintatolimod: a potential treatment in patients with pancreatic cancer expressing Toll-like receptor 3 American journal of cancer research 2023-06-15 [PMID: 37424830] (IHC-P, Human)

Xiao J, Huang J, Jian X et al. IRE1 $\gamma$  arm of unfolded protein response in muscle-specific TGF- $\beta$  signaling-mediated regulation of muscle cell immunological properties Cellular & molecular biology letters 2023-02-27 [PMID: 36849929] (WB, Mouse)

Eskuri M, Kemi N, Helminen O et al. Toll-like receptors 3, 7, 8 and 9 in Gastric Cancer APMIS : acta pathologica, microbiologica, et immunologica Scandinavica 2022-10-22 [PMID: 36271773]

Wolpaw A, Grossmann L, Dong M, et al. Epigenetic state determines inflammatory sensing in neuroblastoma Proc Natl Acad Sci U S A 2022-02-05 [PMID: 35121657]

Helminen O, Huhta H, Lehenkari Petri P. Nucleic acid-sensing Toll-like receptors 3, 7 and 8 in esophageal epithelium, Barrett's esophagus, dysplasia and adenocarcinoma Oncoimmunology 2016-07-29 [PMID: 27467941]

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]

Su R, Cai L, Xiong P et al. TLR3 Expression is a Potential Prognosis Biomarker and Shapes the Immune-Active Tumor Microenvironment in Esophageal Squamous Cell Carcinoma Journal of inflammation research 2022-02-28 [PMID: 35250293] (IF/IHC, Human)

Stolberg-Stolberg J, Boettcher A, Sambale M et al. Toll-like receptor 3 activation promotes joint degeneration in osteoarthritis Cell death & disease 2022-03-11 [PMID: 35277480] (ICC/IF, Human)

Szollosi, A G, McDonald, I Et al. TLR3 in Chronic Human Itch: A Keratinocyte-Associated Mechanism of Peripheral Itch Sensitization. J Invest Dermatol 2019-11-01 [PMID: 31129058] (ICC/IF, Porcine, Mouse, Bovine, Sheep)

More publications at <http://www.novusbio.com/NBP2-24875>



## Procedures

### Immunohistochemistry-Paraffin Protocol for TLR3 Antibody (NBP2-24875)

#### 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.

### Western Blot Protocol for TLR3 Antibody (NBP2-24875)

#### 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.





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### **Products Related to NBP2-24875**

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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)
NBP2-24902	TLR3 Antibody (40C1285.6) [PE]

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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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