# **Product Datasheet**

# TRIF/TICAM1 Antibody - BSA Free NB120-13810

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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# NB120-13810

TRIF/TICAM1 Antibody - 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	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	76 kDa
Product Description	
Host	Rabbit
Gene ID	148022
Gene Symbol	TICAM1
Species	Mouse, Rat, Porcine
Reactivity Notes	Rat reactivity reported in scientific literature (PMID: 23447644). Porcine reactivity reported in scientific literature (PMID: 27046485).
Immunogen	Synthetic peptide made to an internal portion of the mouse TRIF protein (between amino acids 100-180) [UniProt Q80UF7].
Product Application Details	
Applications	Western Blot, Simple Western, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockdown Validated
Recommended Dilutions	Western Blot 1 - 2 ug/ml, Simple Western 1:100, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:10-1:100, Immunoprecipitation reported in scientific literature (PMID 28007523), Immunohistochemistry-Paraffin 1:200, Immunoblotting reported in scientific literature (PMID 27043414), Knockdown Validated
Application Notes	In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See <u>Simple Western Antibody Database</u> for Simple Western validation: Tested in Raji lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:100. Separated by Size-Wes, Sally Sue/Peggy Sue. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.









Western Blot: TRIF/TICAM1 Antibody [NB120-13810] - siRNA was transfected at 50 nM for 48 hrs, and poly(I:C) was added at 100 ug/ml for 4 hrs before cell harvest. Negative control (NC) siRNA served as control. RNAiMAX transfection reagent was used in all the siRNA experiments. The panel shows the knockdown effect of Trif siRNA. Image collected and cropped by CiteAb from the following publication (https://onlinelibrary.wiley.com/doi/full/10.1111/jcmm.13328) licensed under a CC-BY license. Trif GAPD Whole lysate IP:TLR3 TRIF/TICAM1 Antibody [NB120-13810] - Lysates of heart tissue were С immunoprecipitated with anti-TLR3 antibodies (IP: TLR3), followed by IP:lgG MI Sham Sham MI SDS-PAGE and immunoblotting (IB) with indicated antibodies. IP with isotype IgG (IP: IgG) was performed as a control to exclude the non-IB:Trif specific binding of antibodies to cellular proteins. Green arrows indicate non-specific bands. The association between TLR3 and Trif, but not MyD88, was detectable in sham myocardium and was increased in IB:MyD88 infarct. Image collected and cropped by CiteAb from the following publication (https://onlinelibrary.wiley.com/doi/full/10.1111/jcmm.13328) licensed under a CC-BY license. Anti-TLR3 antibody by WB (NB100-IB:TLR3 56571) A TLR3 agonist polyinosinic polycytidylic acid (poly(I:C)) induced MyD88 siRNA **TLR3 siRNA** NC SIRNA NC siRNA SIRNA NC siRNA autophagy in cultured cardiomyocytes through a TRIF dependent pathway. (A) Poly(I:C) increased autophagy markers in cultured H9c2 rat E ventricular cells. (B) Poly(I:C) stimulated autophagosome formation but TLR3 MyD88 Trif did not affect autophagic flux. Primary cultured neonatal rat ventricular GAPDH GAPDH GAPDH myocytes (NRVMs) were transfected with a tandem mRFP GFP LC3 adenovirus for 24 hrs, followed by treatment with poly(I:C) (100 µg/ml, MyD88 siRNA+I:C **TLR3 siRNA+I:C** 4 hrs). Autophagosomes & autolysosomes were, respectively, visualized VC siRNA+I:C **Irif siRNA+I:C** MyD88 siRNA **FLR3 siRNA** as yellow 4 red only punctas under a confocal microscope. (C) An NC siRNA *<b>Irif siRNA* autophagic flux inhibitor chloroquine (CQ) induced accumulations of Vehicle LC3 II & p62/SQSTM1 proteins in H9c2 myocytes receiving poly(I:C) ö (100 µg/ml, 4 hrs). CQ was applied at 10 µM, immediately prior to poly LC3-I (I:C). (D) Effects of indicated siRNA on poly(I:C) □ induced changes in LC3-II autophagy markers in NRVMs. All the siRNAs were transfected at 50 nM p62 for 48 hrs, & poly(I:C) was added at 100 µg/ml for 4 hrs before cell GAPDH harvest. Negative control (NC) siRNA served as control. RNAiMAX transfection reagent was used in all the siRNA experiments. The upper 9 panel shows the knockdown effects of siRNAs, & the lower panel shows LC3-II 6 representative Western blot images (presented from four independent experiments) & densitometry quantitative data (normalized into 'fold of vehicle group'). All quantitative data are expressed as means  $\pm$  S.D. aP < 0.05, AP < 0.01 versus vehicle; bP < 0.05, BP < 0.01 versus poly(I:C). Image collected & cropped by CiteAb from the following publication 2 (https://pubmed.ncbi.nlm.nih.gov/28945004), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



#### **Publications**

Kuan-Yu Lin, Hsin-Yi Yang, Suh-Ching Yang, Ya-Ling Chen, Y. Watanabe, Jiun-Rong Chen Caulerpa lentillifera improves ethanol-induced liver injury and modulates the gut microbiota in rats Current Research in Food Science 2023-07-08 [PMID: 37483276]

R Ko, J Seo, H Park, N Lee, SY Lee Pim1 promotes IFN-beta production by interacting with IRF3 Experimental & Molecular Medicine, 2022-11-29;54(11):2092-2103. 2022-11-29 [PMID: 36446848]

Vogt A, Scull MA, Friling T et al. Recapitulation of the hepatitis C virus life-cycle in engineered murine cell lines Virology 2013-09-01 [PMID: 23777661] (Western Blot, Block/Neutralize)

Ning F, Li X, Yu L et al. Hes1 attenuates type I IFN responses via VEGF-C and WDFY1 Journal of Experimental Medicine 2019-06-03 [PMID: 31015298] (Immunoprecipitation, Western Blot, Block/Neutralize)

Youn SE, Jiang F, Won HY et al. PAUF Induces Migration of Human Pancreatic Cancer Cells Exclusively via the TLR4/MyD88/NF-kappa B Signaling Pathway International journal of molecular sciences 2022-09-27 [PMID: 36232715] (IP, Human)

Karmakar J, Mandal C Interplay Between Sialic Acids, Siglec-E, and Neu1 Regulates MyD88- and TRIF-Dependent Pathways for TLR4-Activation During Leishmania donovani Infection Frontiers in immunology 2021-03-03 [PMID: 33763070] (WB, Mouse)

Ning F, Li X et al. Hes1 attenuates type I IFN responses via VEGF-C and WDFY1. J Exp Med Mar 6 2019 12:00AM [PMID: 31015298] (IP, Human)

Chen YL, Shirakawa H, Lu NS et al. Impacts of fish oil on the gut microbiota of rats with alcoholic liver damage J. Nutr. Biochem. 2020-09-10 [PMID: 32920090] (WB, Rat)

Shi D, Chen M, Liu L et al. Anti-influenza A virus mechanism of three representative compounds from Flos Trollii via TLRs signaling pathways J Ethnopharmacol 2020-01-28 [PMID: 32004628] (WB, Mouse)

Feng Z, Yang R, Wu L et al. Atractylodes macrocephala polysaccharides regulate the innate immunity of colorectal cancer cells by modulating the TLR4 signaling pathway Onco Targets Ther [PMID: 31564895] (Mouse)

Zhou S, Qi Q, Wang X, et al. SjHSP60 induces CD4+ CD25+ Foxp3+ Tregs via TLR4-Mal-drived production of TGFbeta in macrophages. Immunol Cell Biol. 2018-05-21 [PMID: 29697865] (WB, Mouse)

Gao T, Zhang SP, Wang JF et al. TLR3 contributes to persistent autophagy and heart failure in mice after myocardial infarction J. Cell. Mol. Med. 2017-09-25 [PMID: 28945004] (WB, Mouse)

More publications at <u>http://www.novusbio.com/NB120-13810</u>



#### **Procedures**

#### Immunohistochemistry-Paraffin Protocol for TRIF/TICAM1 Antibody (NB120-13810)

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.
- 13. Mount coverslips.

#### Western Blot Protocol for TRIF/TICAM1 Antibody (NB120-13810) 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 NB120-13810

NB820-59670	Mouse Spleen Whole Tissue Lysate (Adult Whole Normal)
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

#### 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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