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Monoclonal Antibody to CD289 / TLR9 - Purified

Alternate names: Toll-like receptor 9, UNQ5798/PRO19605

Catalog No.: AM26253PU-N

Quantity: 0.1 mg

Concentration: 0.1 mg/ml

Background: Toll-like receptors (TLRs) are highly conserved from Drosophila to humans and share structural and functional similarities. TLRs constitute of a family of pattern recognition receptors (PRRs) that mediate cellular responses to a large variety of pathogens (viruses, bacteria, and parasites) by specific recognition of so-called 'pathogen-associated molecular patterns'. Activation of TLRs, a family of at least 11 different members that function either as homo- or heterodimers, leads to activation of NFκB-dependent and IFN-regulatory factor-dependent signaling pathways. TLRs have a central role in innate immunity and are also required for the development of an adaptive immune response. TLRs are expressed by various cells of the immune system, such as macrophages and dendritic cells. They recognize and respond to molecules derived from bacterial, viral and fungal pathogens.
Whereas most TLRs are expressed on the cell surface, TLR9 is expressed intracellularly within one or more endosomal compartments and recognizes nucleic acids. TLR9 detects a rather subtle difference in the DNA of vertebrates compared with that of pathogens. Vertebrate genomic DNAs have mostly methylated CpG dinucleotides where bacterial and viral DNAs have unmethylated CpG dinucleotides. TLR9 undergoes relocation from endoplasmic reticulum to CpG-ODN-containing endosomes. In these endosomes TLR9 becomes a functional receptor after proteolytic cleavage. TLR9 exists as a preformed homodimer and CpG-ODN binding promotes its conformational change, bringing the cytoplasmic TIR-like domains close to each other. This allows a recruitment of the key adapter protein MyD88 which initiates a signalling cascade. The only human immune cell types known to constitutively express TLR9 and to be activated by CpG ODN are pDCs and B cells. TLR9 triggering induces an activation phenotype in the B cells and pDCs, characterized by the expression of costimulatory molecules, resistance to apoptosis, and induces Th1-type immune response profiles.

Uniprot ID: Q9EQU3

NCBI: 10090

GeneID: 81897

Host / Isotype: Mouse / IgG2a

Recommended Isotype Controls: AM03096PU-N

Clone: 5G5

Immunogen: Purified fusion protein of extracellular domain of human TLR9 (AA 1-815) and human IgGFc

Format:
State: Liquid 0.2 µm filtered Ig fraction
Purification: Protein G
Buffer System: PBS
Preservatives: 0.02% sodium azide
Stabilizers: 0.1% bovine serum albumin

Applications:
Immunohistochemistry on frozen sections (7): 10µm sections were fixed with acetone for 10 minutes. PBS washed sections were incubated with 5G5 1:100 in 1% BSA for 30 minutes at RT. (Ref.7). The typical starting working dilution is 1:50.
Immunohistochemistry on paraffin sections (3): Paraffin embedded tissues 5µm sections were made. After antigen retrieval (0.01mol/l, pH6 sodium citrate) and quenching of endogenous peroxidase, sections were blocked with 0.5% ovalbumin and 0.1% gelatin for 20 minutes at RT. Sections were incubated with 5G5 for 1 hour at 37°C (Ref.3). The typical starting working dilution is 1:50.
Flow cytometry (1,5,6,8): RAW264.7 cells were fixed for 15 minutes with 4% formalin and permeabilized (PBS, 0.5%BSA, 0,5% saponin) at RT (Ref.1). The typical starting working dilution is 1:50.
Immunoflourescence (1,3): cells were fixed with 2% formalin for 15 minutes at RT and permeabilized with a mAb (4µg/400µl) containing buffer (PBS, 0.2% BSA, 0.2% saponin) for 1 hour. (Ref.1).
Western blot (1,2,3,4): Reduced lysates were resolved by 10% SDS-PAGE and blotted on nitrocellulose. After blocking with 5% skimmed milk TLR9 was detected with 2µg/ml 5G5 (Ref.1). The typical starting working dilution is 1:50.
Positive control: RAW264.7 macrophages stimulated with IFN.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: The monoclonal antibody 5G5 recognizes human Toll-like receptor 9.

Species Reactivity: **Tested:** Human, canine, mouse

Storage: Store at 2 - 8 °C.
Shelf life: one year from despatch.

General Readings:
1. Ahmad-Nejad P, Häcker H, Rutz M, Bauer S, Vabulas RM, Wagner H. Bacterial CpG-DNA and lipopolysaccharides activate Toll-like receptors at distinct cellular compartments. Eur J Immunol. 2002 Jul;32(7):1958-68. PubMed PMID: 12115616.
2. Rutz M, Metzger J, Gellert T, Luppa P, Lipford GB, Wagner H, et al. Toll-like receptor 9 binds single-stranded CpG-DNA in a sequence- and pH-dependent manner. Eur J Immunol. 2004 Sep;34(9):2541-50. PubMed PMID: 15307186.
3. Rumio C, Besusso D, Palazzo M, Selleri S, Sfondrini L, Dubini F, et al. Degranulation of paneth cells via toll-like receptor 9. Am J Pathol. 2004 Aug;165(2):373-81. PubMed PMID: 15277213.
4. Pratesi G, Petrangolini G, Tortoreto M, Addis A, Belluco S, Rossini A, et al. Therapeutic synergism of gemcitabine and CpG-oligodeoxynucleotides in an orthotopic human pancreatic carcinoma xenograft. Cancer Res. 2005 Jul 15;65(14):6388-93. PubMed PMID: 16024642.
5. Tokumasa N, Suto A, Kagami S, Furuta S, Hirose K, Watanabe N, et al. Expression of Tyk2 in dendritic cells is required for IL-12, IL-23, and IFN-gamma production and the induction of Th1 cell differentiation. Blood. 2007 Jul 15;110(2):553-60. Epub 2007 Mar 29. PubMed PMID: 17395783.
6. Burgener, I et al; Antibodies specific for human or murine Toll-like receptors detect canine leukocytes by flow cytometry. Vet Immunol Immunopathol 2008, 124; 184.
7. Machida H, Ito S, Hirose T, Takeshita F, Oshiro H, Nakamura T, et al. Expression of Toll-like receptor 9 in renal podocytes in childhood-onset active and inactive lupus nephritis.

Nephrol Dial Transplant. 2010 Aug;25(8):2530-537. doi: 10.1093/ndt/gfq058. Epub 2010 Feb 24. PubMed PMID: 20181802.
8. Zheng W, Wang QH, Liu YJ, Liu J, Feng H, Wu JJ, et al. Distinct host-related dendritic cell responses during the early stage of Plasmodium yoelii infection in susceptible and resistant mice. Parasite Immunol. 2010 May;32(5):324-34. doi: 10.1111/j.1365-3024.2009.01190.x. PubMed PMID: 20500661.

Pictures:

THP1 cells were incubated with IgG2a isptype control (A) or α-TLR9 5G5 mAb (B). Cells (140000) were permeabilized with saponin and stained with 0.4 µg 5G5