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Monoclonal Antibody to CD284 / TLR4 - Purified

Alternate names: Toll-like receptor 4

Catalog No.: SM7124P

Quantity: 0.1 mg

Concentration: 0.5 mg/ml

Background: Toll like receptors (TLR) are highly conserved throughout evolution and have been implicated in the innate defense to many pathogens. In Drosophila toll is required for the anti fungal response, while the related 18 wheeler is involved in antibacterial defenses. In mammals, TLR identified as type I transmembrane signaling receptors with pattern recognition capabilities have been implicated in the innate host defense to pathogens. TLR4 has been identified next to MD2 and CD14 as a receptor that is central to the innate immune response to lipopolysaccharides (LPS) of Gram negative bacteria.
TLR4 contributes to the initiation of CNS neuroimmune activation after nerve transection. TLR4 is part of an early, specific, innate CNS/microglial response and maybe relevant in the prevention and treatment of neuropathic pain syndromes. TLR4 is weakly expressed by resting cells, but is upregulated following stimulation with LPS.

Uniprot ID: O00206

NCBI: NM_138554.3

GeneID: 7099

Host / Isotype: Mouse / IgG2b

Recommended Isotype Controls: SM12P, AM03110PU-N

Clone: 76B357.1

Immunogen: A portion of amino acids 100-200 of Human TLR4
Remarks: The amino acid sequence used for immunisation is 100% homologous in Human, Chimp, Baboon, Chinese Hamster, and Rat, 93% homologous in Bovine and Pig, 85% homologous in Sheep and 78% homologous in Cat, Horse and Mouse.

Format:
State: Liquid purified IgG fraction
Purification: Protein G Affinity Chromatography
Buffer System: PBS
Preservatives: 0.05% Sodium Azide
Stabilizers: 0.05% BSA

Applications:
Flow Cytometry (cell surface): 0.5-2 µg/10^6 cells.
Recommended Positive Control: ThP1, Ramos.
Flow Cytometry (Intracellular): See Cohen et al., 2008 for details.
Immunofluorescence: See Nowicki et al, 2009 for details.
Western blot: 1-3 µg/ml.

Recommended Positive Control: Intestine lysate.
Immunohistochemistry on Cryosections: See Nowicki et al, 2012 for details.
Immunohistochemistry on Paraffin Sections: 5 µg/ml.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: The antibody recognizes CD284/TLR4.

Species Reactivity: **Tested:** Human, Mouse, Rat.
Expected from sequence similarity: Horse, Chimpanzee, Pig, Sheep, Bovine and Cat.

Storage: Store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

Product Citations: **Originator or purchased from resellers:**

1. Cohen PA, Koski GK, Czerniecki BJ, Bunting KD, Fu XY, Wang Z, et al. STAT3- and STAT5-dependent pathways competitively regulate the pan-differentiation of CD34pos cells into tumor-competent dendritic cells. Blood. 2008 Sep 1;112(5):1832-43. doi: 10.1182/blood-2007-12-130138. Epub 2008 Jun 24. PubMed PMID: 18577706.
2. Yoshizaki A, Iwata Y, Komura K, Ogawa F, Hara T, Muroi E, et al. CD19 regulates skin and lung fibrosis via Toll-like receptor signaling in a model of bleomycin-induced scleroderma. Am J Pathol. 2008 Jun;172(6):1650-63. doi: 10.2353/ajpath.2008.071049. Epub 2008 May 8. PubMed PMID: 18467694.
3. Johnston MJ, Wang A, Catarino ME, Ball L, Phan VC, MacDonald JA, et al. Extracts of the rat tapeworm, Hymenolepis diminuta, suppress macrophage activation in vitro and alleviate chemically induced colitis in mice. Infect Immun. 2010 Mar;78(3):1364-75. doi: 10.1128/IAI.01349-08. Epub 2009 Dec 22. PubMed PMID: 20028812.
4. Hafez M, Hayes K, Goldrick M, Grencis RK, Roberts IS. The K5 capsule of Escherichia coli strain Nissle 1917 is important in stimulating expression of Toll-like receptor 5, CD14, MyD88, and TRIF together with the induction of interleukin-8 expression via the mitogen-activated protein kinase pathway in epithelial cells. Infect Immun. 2010 May;78(5):2153-62. doi: 10.1128/IAI.01406-09. Epub 2010 Feb 9. PubMed PMID: 20145095.
5. Allam JP, Peng WM, Appel T, Wenghoefer M, Niederhagen B, Bieber T, et al. Toll-like receptor 4 ligation enforces tolerogenic properties of oral mucosal Langerhans cells. J Allergy Clin Immunol. 2008 Feb;121(2):368-374.e1. Epub 2007 Nov 26. PubMed PMID: 18036651.
6. Link A, Selejan S, Maack C, Lenz M, Böhm M. Phosphodiesterase 4 inhibition but not beta-adrenergic stimulation suppresses tumor necrosis factor-alpha release in peripheral blood mononuclear cells in septic shock. Crit Care. 2008;12(6):R159. doi: 10.1186/cc7158. Epub 2008 Dec 17. PubMed PMID: 19091080.
7. Nowicki M, Müller K, Serke H, Kosacka J, Vilser C, Ricken A, et al. Oxidized low-density lipoprotein (oxLDL)-induced cell death in dorsal root ganglion cell cultures depends not on the lectin-like oxLDL receptor-1 but on the toll-like receptor-4. J Neurosci Res. 2010 Feb 1;88(2):403-12. doi: 10.1002/jnr.22205. PubMed PMID: 19705455.
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9. Zanoni G, Navone R, Lunardi C, Tridente G, Bason C, Sivori S, et al. In celiac disease, a subset of autoantibodies against transglutaminase binds toll-like receptor 4 and induces activation of monocytes. PLoS Med. 2006 Sep;3(9):e358. PubMed PMID: 16984219.
10. Brandau S, Jakob M, Hemeda H, Bruderek K, Janeschik S, Bootz F, et al. Tissue-resident mesenchymal stem cells attract peripheral blood neutrophils and enhance their

inflammatory activity in response to microbial challenge. J Leukoc Biol. 2010 Nov;88(5):1005-15. doi: 10.1189/jlb.0410207. Epub 2010 Aug 3. PubMed PMID: 20682625.
11. Weile C, Josefsen K, Buschard K. Glucose activation of islets of Langerhans up-regulates Toll-like receptor 5: possible mechanism of protection. Clin Exp Immunol. 2011 Nov;166(2):251-7. doi: 10.1111/j.1365-2249.2011.04457.x. PubMed PMID: 21985371.
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14. Nowicki M, Kosacka J, Serke H, Blüher M, Spanel-Borowski K. Altered sciatic nerve fiber morphology and endoneural microvessels in mouse models relevant for obesity, peripheral diabetic polyneuropathy, and the metabolic syndrome. J Neurosci Res. 2012 Jan;90(1):122-31. doi: 10.1002/jnr.22728. Epub 2011 Sep 14. PubMed PMID: 21919033.
15. Wu Y, Lu J, Antony S, Juhasz A, Liu H, Jiang G, et al. Activation of TLR4 is required for the synergistic induction of dual oxidase 2 and dual oxidase A2 by IFN-γ and lipopolysaccharide in human pancreatic cancer cell lines. J Immunol. 2013 Feb 15;190(4):1859-72. doi: 10.4049/jimmunol.1201725. Epub 2013 Jan 7. PubMed PMID: 23296709.
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19. Wu J, Meng Z, Jiang M, Zhang E, Trippler M, Broering R, et al. Toll-like receptor-induced innate immune responses in non-parenchymal liver cells are cell type-specific. Immunology. 2010 Mar;129(3):363-74. doi: 10.1111/j.1365-2567.2009.03179.x. Epub 2009 Nov 16. PubMed PMID: 19922426.
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21. McCulloch L, Brown KL, Mabbott NA. Ablation of the cellular prion protein, PrPC, specifically on follicular dendritic cells has no effect on their maturation or function. Immunology. 2013 Mar;138(3):246-57. doi: 10.1111/imm.12031. PubMed PMID: 23121447.
22. Jazaeri F, Tavangar SM, Ghazi-Khansari M, Khorramizadeh MR, Mani AR, Dehpour AR. Cirrhosis is associated with development of tolerance to cardiac chronotropic effect of endotoxin in rats. Liver Int. 2013 Mar;33(3):368-74. doi: 10.1111/liv.12039. Epub 2013 Jan 14. PubMed PMID: 23311391.

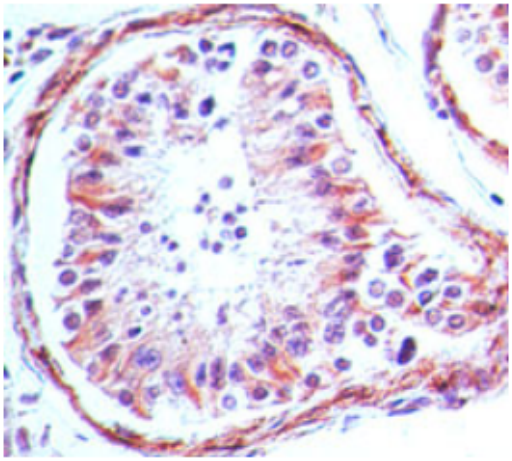
General Readings:

1. Muzio M, Natoli G, Saccani S, Levrero M, Mantovani A. The human toll signaling pathway: divergence of nuclear factor kappaB and JNK/SAPK activation upstream of tumor necrosis factor receptor-associated factor 6 (TRAF6). J Exp Med. 1998 Jun 15;187(12):2097-101. PubMed PMID: 9625770.
2. Medzhitov R, Janeway CA. Innate immunity: the virtues of a nonclonal system of

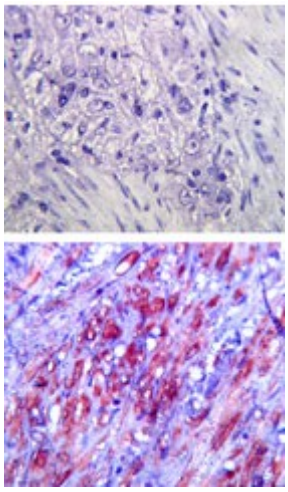
recognition. Cell. 1997 Oct 31;91(3):295-8. PubMed PMID: 9363937.
3. Chuang TH and Ulevitch RJ. Biochim. Biophys. Acta 1518 (1-2): 157-161 (2001).
4. Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, Ogawa T, et al. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity. 1999 Oct;11(4):443-51. PubMed PMID: 10549626.
5. Poltorak A, Ricciardi-Castagnoli P, Citterio S, Beutler B. Physical contact between lipopolysaccharide and toll-like receptor 4 revealed by genetic complementation. Proc Natl Acad Sci U S A. 2000 Feb 29;97(5):2163-7. PubMed PMID: 10681462.
6. Medzhitov R, Preston-Hurlburt P, Janeway CA. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature. 1997 Jul 24;388(6640):394-7. PubMed PMID: 9237759.

Pictures:

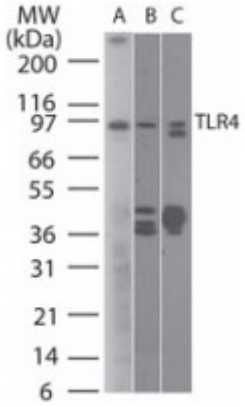
Formalin-Fixed, Paraffin-Embedded Human testis tissue stained with TLR4 Antibody (5 µg/ml).



Immunohistochemical analysis of TLR4 in Paraffin-Embedded Formalin-Fixed Human colon tissue using an isotype control (top) and TLR4 antibody SM7124P (bottom) at 5 µg/ml.



Western blot analysis of TLR4 using TLR4 antibody SM7124P at 2 µg/ml on (A) Human intestine and 6 µg/ml on (B) Mouse intestine and C) Rat intestine lysate.



Flow analysis of cell surface TLR4 in mouse splenocytes using 0.5 µg/10e6 cells of SM7124P. Shaded histogram represents cells without anti-TLR4 antibody, green represents isotype control, red represents anti-TLR4 antibody SM7124P