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Customer Service: 1-800 786 1236  
Technical Service: 214 387 8105  
Fax: 214 387 0870  
[Info@fabgennix.com](mailto:Info@fabgennix.com)  
[www.fabgennix.com](http://www.fabgennix.com)

## Antibodies to Toll Receptor Signaling-Molecules (Toll Receptor)

### Anti- Toll-Receptor 4 Antibodies (Cat # TLR-401AP, FITC-TLR4, P-TLR4 and PC-TLR4)

#### Alternate Nomenclature: Toll like Receptor 4, CD284 antigen,

The mammalian host defense system is essentially regulated by highly conserved Toll-like receptor (TLR) family of proteins. At least 13 TLRs have been identified and cloned in mammalian cells which recognize molecular products/signals from all the major classes of pathogens and activation of innate immunity. The Toll signaling to NF-Kb starts from conserved Toll-IL-1-resistance (TIR) domain, which mediated the coupling of TIR adaptor molecules (MyD88, Mal, TICAM and TRAM) and caused production of inflammatory cytokines such as IL-1, IL-6, IL-8, TNF $\alpha$ , and IL-12, chemokines and co-stimulatory molecules such as CD40, CD80 and CD86. In the presence of inflammatory cytokines and binding of adaptor molecule, MyD88 that binds FADD and triggers apoptosis through the caspase cascade. TLR induced apoptosis pathway appears to be a repertoire of defense mechanism utilized by innate defense mechanism. The constitutive expression of many human TLRs (1, 2, 3) have been shown on the surface of myeloid lineage cells by RT-PCR and use of specific monoclonal antibodies. Upon activation of these receptors by their respective chemokines and ligands have been shown in literature on various cell lines including endothelial, epithelial and other cells. The expression of TLR 3, 7, 8 and 9 are mainly found on endosomal lysosomal compartments. Human TLR3 is expressed in human fibroblasts cells and TLR 9 in in-vitro derived DC cells. There is significant evidence of TLR involvement in many systemic disorders following bacterial infection including sepsis, periodontitis, cardiac ischemia, cerebral palsy and others, understanding the TLRs involvement in these conditions will allow therapeutic interventions at the receptor level for treatment of these disorders.

The TLR are highly conserved protein and share structural and functional domains across species. These receptors recognize pathogen associated molecular patterns (PAMPs) that are expressed on infectious agents, and mediate the production of cytokines needed for production of immediate immunity. TLR4 signaling pathway are associated with expression of pro-inflammatory cytokines and activation of the PI3K/Akt signaling pathway in the hippocampal formation and is involved in ischemia-induced perfusion hippocampal neuronal death (4). Human TLR4 is a 839 amino acid (105 kDa) type I trans-membrane receptor protein characterized by extracellular domains with leucine-rich repeats and a cytoplasmic domain with type I IL-1 receptor. TLR4 deficiency led to the upregulation of what we believe to be a novel NADPH oxidase (Nox), Nox3, in lungs and endothelial cells, resulting in increased oxidant generation and elastolytic activity (5). The TLR 2 and 4 are pattern recognition receptors and signaling molecules in response to bacterial lipoproteins and is involved in innate immunity. TLR4 is expressed on peripheral blood and together with MD-2 and CD14 is responsible for LPS signaling recognition. Many specific adapter molecules (TICAM, MyD88, ICAM2, TRAM, TIRAP, TRIF etc) are also involved in signaling of several other TLRs.

The TLR 4-selective antibodies were generated against peptide from unique region near the C-terminal end of Toll receptor-4 protein, this peptide sequence is not present in other members of the TLR family. FabGennix Inc. has generated rabbit anti-TLR4 mono-epitope-specific antibodies utilizing linear and cyclic peptide methodology. The Anti-TLR4 antibodies have been fully characterized for cross reactivity with other members of the TLR family molecules and with cellular proteins using Western blot analyses. The TLR4 antibody is also available as FITC-conjugate, other conjugates can be ordered at a nominal charge. FabGennix International Inc., has produced antibodies to all Toll receptors (TLR1-TLR13), these antibodies are now available for sale from us or from our distributors. FabGennix Int. Inc., also provide western blot positive controls for TLR4 in ready-to-use buffer. Limited quantities of the antigenic blocking peptide for TLR4 antibodies is also available (inquire for availability).

Catalog #	Host	Description	Antigen/ control	Cross reactivity	Quantity
TLR-401AP	Rabbit	Affinity purified Toll Receptor 3 Antibody	Peptide antibody	H	100 ug
FITC-TLR4	Rabbit	Affinity purified FITC conjugated TLR4 antibody	Peptide antibody	n/a	250 ug
P-TLR4	n/a	Antigenic blocking peptide for TLR4 antibody	Synthetic peptide	n/a	250 ug
*PC-TLR4	n/a	Western blot positive control for TLR4	Ready-to-use WB control for TLR4	Human/inquire	For 5 appl

R = rat; M = mouse; H = humans; R = rabbit. WB, Western Blot analyses; IMM, Immunoprecipitation; IHC, Immunohistochemistry, n.d, not determine.

**Immunogen:** Synthetic peptides are selected from human Toll receptor 4 (TLR4) corresponding to amino acids 976-998 (dkg sw n peg tv g tgc n w q eat si), the peptide was post-synthetically modified to achieve desired antigenicity before coupling to a carrier protein.

**Concentration:** TLR-401AP antibody has Ig concentration ranging from 0.66-0.80mg/ml in antibody stabilization buffer.

**Applications:** For ELISA/Dot blot assay the antibody TLR-401AP can be diluted to 1:20,000 in diluOBuffer (Cat # FGI-1963). For Western blot applications starting dilution of 1:500 in DiluOBuffer (Cat # FGI-1963) is recommended. Other applications and species cross reactivity for this antibody is yet to be determined.

**Reactivity:** The antibody TLR-401AP labels a band of around 104 kDa in PC-TLR4 samples. The Western on the left is a 10% gel with antibody TLR-401AP diluted 1:500 in diluOBuffer.

**Protocols:** Standard protocol for various applications (Western blot; immunoprecipitation and immunohistochemistry) of this antibody can be obtained by calling technical service hotline. The general information on this antibody is supplied with the product. FabGennix Inc. recommends investigators to optimize conditions for optimal usage of this product.

**Form/Storage:** The antiserum is supplied in antibody stabilization buffer with preservatives. For long-term storage of antibody, store at -20°C FabGennix Inc. does not recommend storage of very dilute antibody solutions unless they are prepared in specially formulated multi-use antibody dilution buffer (Cat # DiluOBuffer). Working solutions of antibodies in DiluOBuffer should be filtered through 0.45um filter after every use for long-term storage.



Western Blot of TLR3. The TLR4 antibody (Cat # TLR-401AP) at 1:500 in diluOBuffer was probed with PC-TLR4 sample. MW of TLR4 is approximately 104 kDa.

**Notes:** Briefly centrifuge to collect liquid, heat or boil PC-TLR4 tube for 1-2 minutes to dissolve any precipitate before use. This product is "ready-to-use" for electrophoresis. After thawing store at room temperature. Repeated freezing and thawing may result in appearance of higher MW immunoreactive bands.

**New Reagents:** Now you can recycle your western blots (nitrocellulose, supported membranes and PVDF membranes) by using our StripOBuffer (Cat FGI-1989). Each stripping is guaranteed to give better signal (up to 8 stripping). No strong pungent smell of reducing agents or heating required. Block in 5X diluOBuffer and you are ready for blotting with a new antibody

#### References:

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- Xuchen Zhang, Peiyang Shan, Ge Jiang, Lauren Cohn, and Patty J. Lee. Toll-like receptor 4 deficiency causes pulmonary emphysema. J Clin Invest. 2006 November 1; 116(11): 3050-3059.

\* For users who may require large amounts of TLR-401AP, please inquire about bulk material discounts.  
This Product is for Research Use Only and is NOT intended for use in humans or clinical diagnosis.

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5850 Town and Country Blvd, Suite 301, Frisco, TX 75034  
Customer service: 1800 786 1236; Technical Support: 214 387 8105