Human TLR4 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1478

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human TLR4 in direct ELISAs and Western blots. In direct ELISAs and Western blots, approximately 30% cross-reactivity with recombinant mouse (rm) TLR4 is observed and less than 5% cross-reactivity with recombinant human (rh) TLR1, rhTLR2, rhTLR3 and rmTLR6 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human TLR4 Glu24-Lys631 Accession # O00206
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

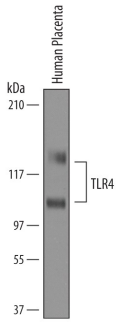
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Flow Cytometry	2.5 µg/10^6 cells	Human peripheral blood monocytes
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Neutralization	Measured by its ability to neutralize LPS-induced IL-8 secretion in the HEK293 human embryonic kidney cell line co-transfected with human TLR4 and MD-2. The Neutralization Dose (ND_{50}) is typically 1.5-7.5 µg/mL in the presence of 75 ng/mL Lipopolysacharide (LPS).	

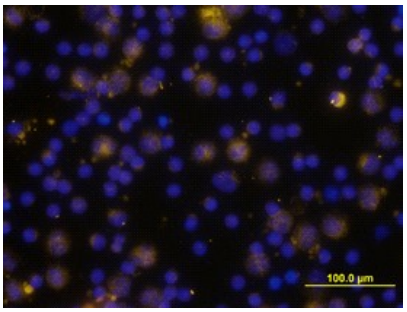
DATA

Western Blot



Detection of Human TLR4 by Western Blot. Western blot shows lysates of human placenta tissue. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human TLR4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1478) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). Specific bands were detected for TLR4 at approximately 110 kDa and 130 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 8.

Immunocytochemistry



TLR4 in Human PBMCs. TLR4 was detected in immersion fixed human peripheral blood mononuclear cells (PBMCs) using Goat Anti-Human TLR4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1478) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (yellow; Catalog # NL001) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

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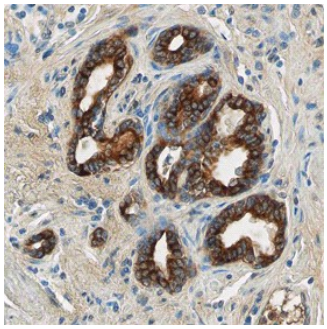
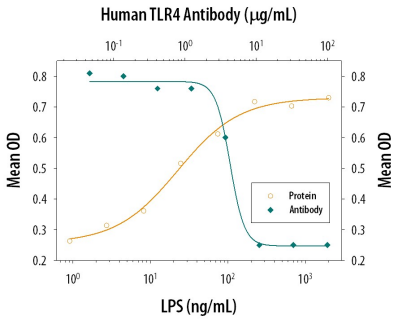
Immunohistochemistry



TLR4 in Human Prostate. TLR4 was detected in immersion fixed paraffin-embedded sections of human prostate using Goat Anti-Human TLR4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1478) at 10 µg/mL overnight at 4 °C. Before incubation with the primary antibody tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Neutralization



IL-8 Secretion Induced by LPS and Neutralization by Human TLR4 Antibody. Lipopolysacharide (LPS) stimulates IL-8 secretion in the HEK293 human embryonic kidney cell line co-transfected with human TLR4 and MD-2, in a dose-dependent manner (orange line), as measured by the Human CXCL8/IL-8 Quantikine ELISA Kit (Catalog # D8000C). IL-8 secretion elicited by LPS (75 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Human TLR4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1478). The ND_{50} is typically 1.5-7.5 µg/mL.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

TLR4 is a 100 kDa type I transmembrane glycoprotein that belongs to the mammalian Toll-Like Receptor family of pathogen pattern recognition molecules. In the literature molecular weights correspondent to 110 kDa and 130 kDa were reported for TLR4 (1). MD-2, also known as ESOP-1, is a 25 kDa secreted protein that is required for TLR4-mediated responses to bacterial lipopolysaccharide (LPS) (1-4). The human TLR4 cDNA encodes an 839 amino acid (aa) precursor that contains a 23 aa signal sequence, a 608 aa extracellular domain (ECD), a 21 aa transmembrane segment, and a 187 aa cytoplasmic domain. TLR4 contains 21 leucine rich repeats in its ECD and one cytoplasmic Toll/IL-1 receptor (TIR) domain (5). The ECD of human TLR4 shares approximately 25% aa sequence identity with other TLRs and 60%-74% aa sequence identity with bovine, equine, feline, mouse, rat, and porcine TLR4. The human MD-2 cDNA encodes a 160 aa precursor with an 18 aa signal sequence (5). Human MD-2 shares 20% aa sequence identity with MD-1 and 62%-64% aa sequence identity with bovine, mouse, and rat MD-2. MD-2 associates with TLR4 on monocytes, macrophages, dendritic cells, and B cells (6-8). MD-2 expression is required for cell surface localization of TLR4 and for optimal LPS-induced TLR4 signaling (8, 9). MD-2 also forms soluble disulfide-linked homo-oligomers which can interact with TLR4 (7). Through a domain separate from its TLR4-binding domain, MD-2 extracts LPS from circulating CD14-LPS complexes and carries the LPS into a ternary complex with TLR4 (10-12). The interaction of MD-2/LPS with TLR4 induces receptor oligomerization and the triggering of an inflammatory response (13). Increased levels of plasma MD-2 in septic shock patients sensitizes MD-2 non-expressing epithelial cells to LPS and promotes widespread tissue inflammation (14).

References:

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