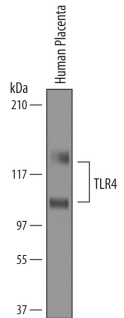
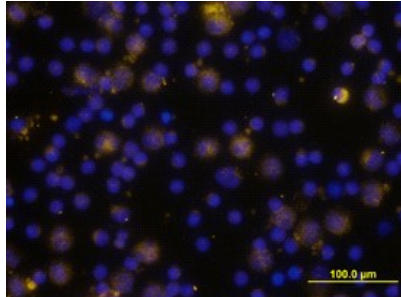
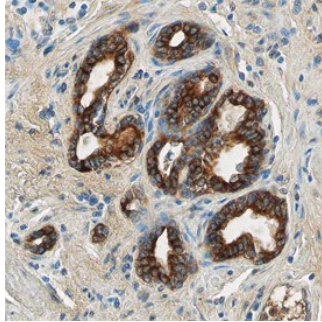


DESCRIPTION	
Species Reactivity	Human
Specificity	Detects human TLR4 in direct ELISAs and Western blots. In direct ELISAs and Western blots, approximately 30% cross-reactivity with recombinant mouse (rm) TLR4 is observed and less than 5% cross-reactivity with recombinant human (rh) TLR1, rhTLR2, rhTLR3 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 as a 0.2 µm filtered solution in PBS.

APPLICATIONS		
Please Note: Optimal dilutions should be determined by each laboratory for each application. <i>General Protocols</i> are available in the <i>Technical Information</i> 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 Lipopolysaccharide (LPS).	

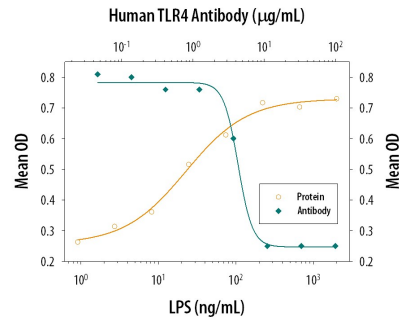
DATA	
<p>Western Blot</p>  <p>Detection of Human TLR4 by Western Blot. Western blot shows lysates of human placenta tissue. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human TLR4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1478) followed by HRP-conjugated Anti-Goat IgG Secondary 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.</p>	<p>Immunocytochemistry</p>  <p>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.</p>

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. Lipopolysaccharide (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 # D800C). 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 ND₅₀ 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. <ul style="list-style-type: none"> • 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).

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