

#### DESCRIPTION

<b>Species Reactivity</b>	Human
<b>Specificity</b>	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.
<b>Source</b>	Polyclonal Goat IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>	Mouse myeloma cell line NS0-derived recombinant human TLR4 Glu24-Lys631 Accession # O00206
<b>Endotoxin Level</b>	<0.10 EU per 1 µg of the antibody by the LAL method.
<b>Formulation</b>	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.

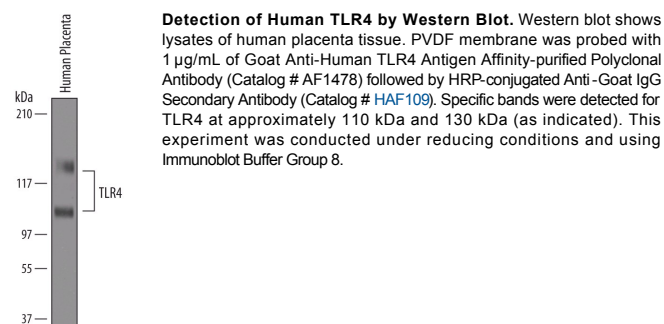
#### APPLICATIONS

**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.

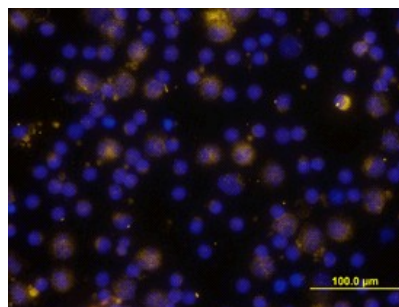
	Recommended Concentration	Sample
<b>Western Blot</b>	1 µg/mL	See Below
<b>Flow Cytometry</b>	2.5 µg/10 <sup>6</sup> cells	Human peripheral blood monocytes
<b>Immunocytochemistry</b>	5-15 µg/mL	See Below
<b>Immunohistochemistry</b>	5-15 µg/mL	See Below
<b>Neutralization</b>	Measured by its ability to 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 <sub>50</sub> ) is typically 1.5-7.5 µg/mL in the presence of 75 ng/mL Lipopolysaccharide (LPS).	

#### DATA

##### Western Blot

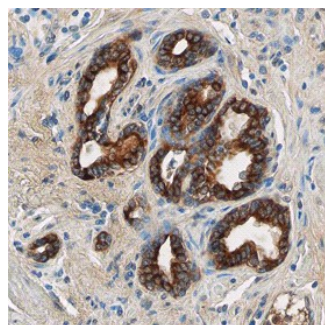


##### Immunocytochemistry



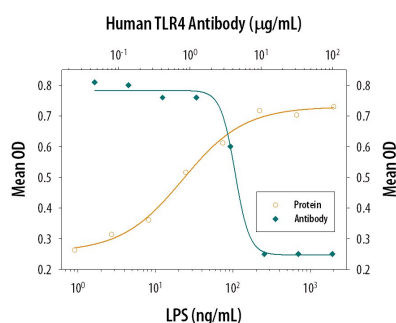
**TLR4 in Human PBMCs.** TLR4 was detected in immersion fixed human peripheral blood mononuclear cells (PBMCs) using Goat Anti-Human TLR4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1478) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated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

##### 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 # 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<sub>50</sub> is typically 1.5-7.5 µg/mL.

#### PREPARATION AND STORAGE

<b>Reconstitution</b>	Reconstitute at 0.2 mg/mL in sterile PBS.
<b>Shipping</b>	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"><li>● 12 months from date of receipt, -20 to -70 °C as supplied.</li><li>● 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li><li>● 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li></ul>

#### 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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