# CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

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|---|---|---|---|
| **Product name** | TLR4, Human, clone HTA125, R-PE labeled | | |
| **Catalog number** | HM2068PE-100 | | |
| **Lot number** | - | **Expiry date** | - |
| **Volume** | 1 ml | **Amount** | 100 tests |
| **Formulation** | 0.2 µm filtered in PBS+1%BSA+0.02%NaN3 | **Concentration** | - |
| **Host Species** | Mouse IgG2a | **Conjugate** | R-PE |
| **Endotoxin** | N.A. | **Purification** | Protein G |
| **Storage** | 4°C | | |

## Application notes

| | IHC-F | IHC-P | IF | FC | FS | IA | IP | W |
|---|---|---|---|---|---|---|---|---|
| Reference # | | | 6 | 1,3,8 | 2,3,4,9 | 5 | 1 | 7 |
| Yes | | | ● | ● | ● | ● | ● | ● |
| No | | | | | | | | |
| N.D. | ● | ● | | | | | | |

*N.D.= Not Determined; IHC = Immuno histochemistry; F = Frozen sections; P = Paraffin sections; IF = Immuno Fluorescence; FC = Flow Cytometry; FS = Functional Studies; IA = Immuno Assays; IP = Immuno Precipitation; W = Western blot*

Dilutions to be used depend on detection system applied. It is recommended that users test the reagent and determine their own optimal dilutions.

- FC: 300000 cells/50µl were stained with 2 µg antibody for 30 minutes at 4°C
- FS: In cell culture 10 µg/ml
- IF: Oregon green labeled HTA125 was used in FRAP measurements
- IP: HTA125 (4 mg/ml) coupled to Sepharose 4FF beads was added to cell lysate and incubated for 2 hours at 4°C
- W: 20 mg protein was analyzed on SDS-PAGE and transferred to nitrocellulose. Blot was blocked with TBS/5% dry milk/0.1% tween-20
- Positive control: Macrophages; Negative control: HEK293 cells.

## General Information

**Description**

Toll-like receptors (TLRs) are highly conserved from Drosophila to humans and share structural and functional similarities. TLRs constitute of a family of pattern recognition receptors (PRRs) that mediate cellular responses to a large variety of pathogens (viruses, bacteria, and parasites) by specific recognition of so-called 'pathogen-associated molecular patterns'. Activation of TLRs, a family of at least 11 different members that function either as homo- or heterodimers, leads to activation of NFκB-dependent and IFN-regulatory factor-dependent signaling pathways. TLRs have a central role in innate immunity and are also required for the development of an adaptive immune response. TLRs are expressed by various cells of the immune system, such as macrophages and dendritic cells. TLRs are class I receptors, with a single α-helix that spans the cell membrane. They recognize and respond to molecules derived from bacterial, viral and fungal pathogens, such as lipopolysaccharide (LPS) from the outer membrane of Gram negative bacteria, peptidoglycan fragments from bacterial cell walls and single-stranded and double-stranded RNA from viruses. Toll-like receptor 4 (TLR4; CD284) has been identified, next to MD-2 and CD14, as a receptor that is central to the innate immune response to LPS of Gram-negative bacteria. TLR4 is unique among TLRs in its ability to activate two distinct signaling pathways; one pathway is activated by the adaptors TIRAP (Toll/interleukin-1- receptor (TIR)-domain-containing adaptor protein) and MyD88, which leads to the induction of pro-inflammatory cytokines. The second pathway is activated by the adaptors TRIF (TIR-domaincontaining adaptor protein inducing interferon-β) and TRAM (TRIFrelated adaptor molecule), which leads to the induction of type I interferons. The monoclonal antibody HTA125 is a TLR4 function-blocking antibody. HTA125 recognizes preferentially human TLR4 that is associated with MD-2.

**Immunogen**

BALB/c mice were immunized with the Ba/F3 line expressing TLR4.

**Aliases**

Toll-Like receptor 4, TLR4, CD284, ARMD10

**Cross reactivity**

Canine: Yes; Cynomolgus monkey: Yes; Rhesus monkey: Yes; Marmoset monkey: Yes.

**References**


1. Shimazu, R et al; MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. J Exp Med 1999, *189*: 1777
2. Tabeta, K et al; Toll-like receptors confer responsiveness to lipopolysaccharide from porphyromonas gingivalis in human gingival fibroblasts. Infect Immun 2000, *68*: 3731

3. Akashi, S et al; Regulatory roles for CD14 and phosphatidylinositol in the signaling via Toll-like receptor 4-MD-2. Biochem Biophys Res Commun 2000, *268*: 172
4. Wang, J et al; Involvement of CD14 and Toll-like receptors in activation of human monocytes by Aspergillus fumigatus hyphae. Infect Immun 2001, *69*: 2402
5. Walton, K et al; Receptors involved in the oxidized 1-palmitoyl-2 arachidonoyl-sn-glycero-3-phosphorylcholine-mediated synthesis of interleukin-8. J Biol Chem 2003, *278*: 29661
6. Triantafilou, M et al; Lateral diffusion of Toll-like receptors reveals that they are transiently confined within lipid rafts on the plasma membrane. J Cell Sci 2004, *117*: 4007
7. Elner, S et al; TLR4 mediates human retinal pigment epithelial endotoxin binding and cytokine expression. Trans Am Ophthalmol Soc 2005, *103*: 126
8. Burgener, I et al; Antibodies specific for human or murine Toll-like receptors detect canine leukocytes by flow cytometry. Vet Immunol Immunopathol 2008, *124*: 184
9. Brüll, F et al; TLR2 activation is essential to induce a Th1 shift in human peripheral blood mononuclear cells by plant stanols and plant sterols. J Biol Chem 2010, *285*: 2951
10. Stribos, E et al; Renal expression of Toll-like receptor 2 and 4: Dynamics in human allograft injury and comparison to rodents. Molecular Immunology 2015, *64*: 82

**Storage&stability** Product should be stored at 4°C. Under recommended storage conditions, product is stable for at least one year.

**Precautions** For research use only. Not for use in or on humans or animals or for diagnostics. It is the responsibility of the user to comply with all local/state and federal rules in the use of this product. Hycult Biotech is not responsible for any patent infringements that might result from the use or derivation of this product.

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We hereby certify that the above-stated information is correct and that this product has been successfully tested by the Quality Control Department. This product was released for sale according to the existing specifications. This document has been produced electronically and is valid without a signature.

| Approved by Manager of QC | Date |
|---|---|
| Robbert Zwinkels | 16/03/2018 |

Do you have any questions or comments regarding this product? Please contact us via support@hycultbiotech.com.