

# PAb hTLR4

A polyclonal antibody specific for human TLR4

Catalog code: pab-hstlr4

<https://www.invivogen.com/pab-hstlr4>

For research use only, not for diagnostic or therapeutic use

Version 21D20-MM

## PRODUCT INFORMATION

### Contents

- 200 µg polyclonal anti-hTLR4 antibody (PAb-hTLR4), provided azide-free and lyophilized.

**Isotype:** Rat IgG

**Formulation:** H<sub>2</sub>O with 250 U/ml Pen and 250 µg/ml Strep

### Antibody resuspension

Add 1 ml of sterile PBS to obtain a concentration of 0.2 mg/ml.

### Storage and stability

- Product is shipped at room temperature. Lyophilized PAb-hTLR4 should be stored at -20 °C. Lyophilized product is stable for 1 year when properly stored.

- Resuspended PAb-hTLR4 should be stored at -20 °C for 1 year.

### Quality control

- The antibody has been validated for neutralization using cellular assays.

- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

## DESCRIPTION

PAb hTLR4 is a polyclonal antibody specific for human Toll-like receptor 4 (TLR4, CD284). PAb hTLR4 was generated by DNA vaccination. Wistar rats received four hydrodynamic injections of pVAC-hTLR4, a plasmid expressing the extracellular region of human TLR4. The sera were harvested and the IgG fraction purified by Protein G affinity chromatography.

## BACKGROUND

TLR4, the first human TLR identified, is the receptor for Gram-negative lipopolysaccharide (LPS). The TLR4 gene was shown to be mutated in C3H/HeJ and C57BL/10ScCr mice, both of which are low responders to LPS<sup>1</sup>. However, TLR4 alone is not sufficient to confer LPS responsiveness. TLR4 requires MD-2, a secreted molecule, to functionally interact with LPS<sup>2</sup>. Furthermore, a third protein, called CD14, was shown to participate in LPS signaling, leading to NF-κB translocation. This signaling is mediated through several adaptor proteins: MyD88 TIRAP/Mal<sup>3</sup>, TRIF/TICAM1 and TRAM/TICAM2<sup>4</sup>.

1. Poltorak A. *et al.*, 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science*, 282(5396):2085-8. 2. Shimazu R. *et al.*, 1999. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *J Exp Med*, 189(11):1777-82. 3. Horng T. GM. Barton, and R. Medzhitov, 2001. TIRAP: an adapter molecule in the Toll signaling pathway. *Nat Immunol*, 2(9):835-41. 4. Fitzgerald KA. *et al.*, 2003. LPS-TLR4 Signaling to IRF-3/7 and NF-κB Involves the Toll Adaptors TRAM and TRIF. *J Exp Med*. 198(7):1043-1055.

## APPLICATIONS

PAb hTLR4 can be used for neutralization of TLR4, it blocks LPS-induced cellular activation. Other applications have not been tested.

## METHOD

### TLR4 neutralization using PAb-hTLR4

Neutralization experiments were performed in THP1-XBlue™-MD2-CD14 cells which derive from the human monocytic THP-1 cell line. This cell line naturally expresses many pattern-recognition receptors, including TLR4. THP1-XBlue™-MD2-CD14 cells were stably transfected with the human MD2 and CD14 genes, in addition to an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. Hence, TLR4 stimulation of THP1-XBlue™-MD2-CD14 cells induces SEAP production following the activation of NF-κB. The amount of SEAP secreted in the supernatant of these cells can be readily detected when using QUANTI-Blue™ Solution, a SEAP detection medium. QUANTI-Blue™ Solution will turn blue following TLR stimulation but remain pink if neutralization occurs.

1. Prepare a 1/10 PAb-hTLR4 dilution (20 µg/ml) using culture medium containing heat inactivated fetal bovine serum (FBS).

*Note: Some lots of FBS contain endogenous alkaline phosphatase that can interfere with SEAP.*

2. Prepare a cell suspension at 1x10<sup>6</sup> cells/ml.

3. Add 100 µl of PAb-hTLR4 dilution (5 µg/ml final concentration).

4. Add 100 µl of cell suspension per well of a 96-well plate.

5. Incubate for 1 hour at 37 °C.

6. Add 50 µl of LPS (10-100 ng/ml final concentration).

7. Incubate 8-24 hours at 37 °C

8. Add 20 µl supernatant to 180 µl QUANTI-Blue™ Solution in a 96-well plate.

9. Incubate 1- 6 hours at 37 °C

10. Assess SEAP levels with the naked eye or spectrophotometrically by reading the OD at 655 nm.

## RELATED PRODUCTS

Product	Catalog Code
LPS-EB ultrapure ( <i>E. coli</i> O111:B4)	tlrl-3pelps
LPS-EK ultrapure ( <i>E. coli</i> K12)	tlrl-pekpls
LPS-SM ultrapure ( <i>S. minnesota</i> )	tlrl-smlps
Monophosphoryl Lipid A (MPLA)	tlrl-mpla
QUANTI-Blue™ Solution	rep-qbs

## TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Asia: +852 3-622-34-80

E-mail: [info@invivogen.com](mailto:info@invivogen.com)