PAb hTLR2

Polyclonal antibody to human TLR2

Catalog # pab-hstlr2

For research use only, not for diagnostic or therapeutic use

Version # 07E15-MT

PRODUCT INFORMATION

<u>Content</u>

 \bullet 200 μg polyclonal anti-hTLR2 antibody (PAb-hTLR2), provided sterile, azide-free and lyophilized.

Isotype: Rat IgG Formulation: H₂O with 250 U/ml Pen and 250 μ g/ml Strep

Antibody resuspension

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

Storage

• Product is shipped at room temperature. Lyophilized PAb-hTLR2 should be stored at -20°C. Product is stable for 1 year.

• Resuspended PAb-hTLR2 should be stored at -20°C for 1 year.

Description

.PAb hTLR2 is a polyclonal antibody specific for human Toll-like receptor 2 (TLR2, CD282). PAb hTLR2 was generated by DNA vaccination. Wistar rats received four hydrodynamic injections of pVAC-hTLR2, a plasmid expressing the extracellular region of human TLR2. The sera were harvested and the IgG fraction purified by Protein G affinity chromatography.

BACKGROUND

TLR2 is involved in the recognition of a wide array of microbial molecules. TLR2 recognizes lipoteichoic acid and lipoprotein from gram-positive bacteria, lipoarabinomannan from mycobacteria, and zymosan from yeast cell wall. Moreover, TLR2 participates in the recognition of some types of LPS. TLR2 is known to heterodimerize with other TLRs, a property believed to extend the range of microbial molecules that TLR2 can recognize. TLR2 cooperates with TLR6 in response to diacylated mycoplasmal lipopeptide¹, and associates with TLR1 to recognize triacylated lipopetides². Furthermore, pathogen recognition by TLR2 is strongly enhanced by CD14³.

References

 Girard R *et al.*, 2003. Lipopolysaccharides from Legionella and Rhizobium stimulate mouse bone marrow granulocytes via Toll-like receptor 2. J Cell Sci. 116(Pt 2):293-302.
Ozinsky A. *et al.*, 2000. The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. Proc Natl Acad Sci USA. 97(25):13766-71.

 Lotz S. *et al.*, 2004. Highly purified lipoteichoic acid activates neutrophil granulocytes and delays their spontaneous apoptosis via CD14 and TLR2. J Leukoc Biol. 75(3):467-77.
Schindler U. & Baichwal VR., 1994. Three NF-κB binding sites in the human Eselectin gene required for maximal tumor necrosis factor alpha-induced expression. Mol Cell Biol, 14(9):5820-5831.

APPLICATIONS

PAb hTLR2 can be used for neutralization of TLR2, it blocks the cellular activation of TLR2 induced by agonists such as Pam3CSK4 and FSL-1. Other applications have not been tested.

Neutralization Protocol

Neutralization experiments were performed in THP1 cells, a human monocytic cell line that naturally expresses TLR2, and HEK293 cells transfected to stably express human TLR2. These cells were further transfected with pNiFty-SEAP, a plasmid that expresses a secreted embryonic alkaline phosphatase (SEAP) gene under the control of an NF-κB-inducible ELAM-1 (E-selectin) promoter⁴. The amount of SEAP secreted in the supernatant can be readily detected when using QUANTI-Blue[™], a SEAP detection medium. QUANTI-Blue[™] will turn blue following TLR stimulation but remain pink if neutralization occurs.

Procedure for HEK293/TLR2-SEAP cells

1- Prepare a 1/10 PAb-hTLR2 dilution (20 μ g/ml) using culture medium with heat inactivated FBS.

<u>Note:</u> Some lots of FBS contain endogenous alkaline phosphatase that can interfere with SEAP.

2- Prepare a cell suspension at 250,000 cells/ml.

- 3- Add 100 µl of cell suspension per well of a 96-well plate.
- 4- Add 100 µl of PAb-hTLR2 dilution (5 µg/ml final).
- 5- Incubate 10 min at 37°C.
- 6- Add 5 ng/ml of Pam3CSK4 or FSL-1.
- 7- Incubate overnight at 37°C
- 8- Add 50 µl supernatant to 150 µl QUANTI-Blue[™] in a 96-well plate. 9- Incubate 15-30 min at 37°C

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

RELATED PRODUCTS

MAb hTLR2 (monoclonal) mab-htlr2	
295 -http://pUNO-hTLR2 (human gene) 295 -http://puno-htlr2 $pNiFty-SEAP$ $pnifty-seap$ $QUANTI-Blue^{TM}$ $rep-qb-1$ $Pam3CSK4$ $tlrl-pms$ $FSL-1$ $tlrl-fsl$ $TLR2$ Agonist Kit $tlrl-kit2$	



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