

PAb hTLR6

Polyclonal antibody to human TLR6

Catalog # pab-hstlr6

For research use only, not for diagnostic or therapeutic use

Version # 07E15-MT

PRODUCT INFORMATION

Content

• 200 µg polyclonal anti-hTLR6 antibody (PAb-hTLR6), provided sterile, azide-free and lyophilized.

Isotype: Rat IgG

Formulation: H₂O 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

- Product is shipped at room temperature. Lyophilized PAb-hTLR6 should be stored at -20°C. Product is stable for 6 months.
- Resuspended PAb-hTLR6 should be stored at 4°C for 1 month or at -20°C for 3 months.

Description

PAb hTLR6 is a polyclonal antibody specific for human Toll-like receptor 6 (TLR6). PAb hTLR6 was generated by DNA vaccination. Wistar rats received four hydrodynamic injections of pVAC-hTLR6, a plasmid expressing the extracellular region of human TLR6. The sera were harvested and the IgG fraction purified by Protein G affinity chromatography.

BACKGROUND

TLR6, which is closely related to TLR1, is expressed predominantly in spleen, thymus, ovary and lung¹. TLR6 cooperates with TLR2 to recognize diacylated lipoproteins (LPs) found in mycoplasma but is not required in the recognition of triacylated LPs found in bacteria². This suggests that TLR6 is able to discriminate between the N-terminal lipoylated structures of LPs derived from mycoplasma and bacteria. TLR6 collaboration with TLR2 is essential for the recognition and signal transduction of diacylated LPs, such as MALP-2 and FSL-1^{2,3}, as well as heat-killed mycoplasma, indicating that TLR6 and TLR2 play a central role in recognizing mycoplasma².

References

1. Takeuchi O. *et al.*, 1999. TLR6: A novel member of an expanding toll-like receptor family. *Gene*. 231(1-2):59-65.
2. Takeuchi O. *et al.*, 2001. Discrimination of bacterial lipoproteins by Toll-like receptor 6. *Int Immunol*, 13(7):933-40.
3. Fujita M. *et al.*, 2003. Involvement of leucine residues at positions 107, 112, and 115 in a leucine-rich repeat motif of human Toll-like receptor 2 in the recognition of diacylated lipoproteins and lipopeptides and Staphylococcus aureus peptidoglycans. *J Immunol*. 171(7):3675-83.
4. Schindler U. & Baichwal VR., 1994. Three NF-κB binding sites in the human E-selectin gene required for maximal tumor necrosis factor alpha-induced expression. *Mol Cell Biol*, 14(9):5820-5831.

APPLICATIONS

PAb hTLR6 can be used for neutralization of TLR6, it blocks the cellular activation of TLR6 induced by TLR2/TLR6 agonists, such as 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 TLR6, and HEK293 cells (which express TLR6 endogenously) transfected to stably express human TLR2. These cells were further transfected with pNiFty-SEAP, a plasmid that expresses a secreted embryonic alkaline phosphatase (SEAP) 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-hTLR6 dilution (20 µg/ml) using culture medium with heat inactivated FBS.
- Note: 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-hTLR6 dilution (5 µg/ml final).
- 5- Incubate 10 min at 37°C.
- 6- Add 5 ng/ml of 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

Product	Catalog Code
PAb hTLR2 (polyclonal)	pab-htlr2
293/hTLR2	293-htlr2
pUNO-hTLR2 (human gene)	puno-htlr2
pUNO-hTLR6 (human gene)	puno-htlr6
pNiFty-SEAP	pnifty-seap
QUANTI-Blue™	rep-qb-1
FSL-1	tlrl-fsl

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