

# PAb hTLR5

## Polyclonal antibody to human TLR5

Catalog # pab-hstlr5

For research use only, not for diagnostic or therapeutic use

Version # 07E15-MT

### PRODUCT INFORMATION

#### Content

• 200 µg polyclonal anti-hTLR5 antibody (PAb-hTLR5), provided sterile, 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

- Product is shipped at room temperature. Lyophilized PAb-hTLR5 should be stored at -20°C. Product is stable for 1 year.
- Resuspended PAb-hTLR5 should be stored at -20°C for 1 year.

#### Description

PAb hTLR5 is a polyclonal antibody specific for human Toll-like receptor 5 (TLR5). PAb hTLR5 was generated by DNA vaccination. Wistar rats received four hydrodynamic injections of pVAC-hTLR5, a plasmid expressing the extracellular region of human TLR5. The sera were harvested and the IgG fraction purified by Protein G affinity chromatography.

### BACKGROUND

TLR5 recognizes flagellin from both Gram-positive and Gram-negative bacteria. Activation of the receptor stimulates the production of proinflammatory cytokines, such as TNF- $\alpha$ , through signaling via the adaptor protein MyD88 and the serine kinase IRAK<sup>1,2</sup>. TLR5 can generate a proinflammatory signal as a homodimer suggesting that it might be the only TLR participating in flagellin recognition<sup>2</sup>. However, TLR5 may require the presence of a co-receptor or adaptor molecule for efficient ligand recognition and/or signaling<sup>3</sup>.

#### References

1. Gewirtz AT, *et al.*, 2001. Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. *J Immunol*, 167(4):1882-5.
2. Hayashi F, *et al.*, 2001. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature*, 410(6832):1099-103.
3. Tallant T, *et al.*, 2004. Flagellin acting via TLR5 is the major activator of key signaling pathways leading to NF-kappa B and proinflammatory gene program activation in intestinal epithelial cells. *BMC Microbiol*. 4(1):33.
4. Schindler U. & Baichwal VR., 1994. Three NF- $\kappa$ B 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 hTLR5 can be used for neutralization of TLR5, it blocks flagellin-induced cellular activation. Other applications have not been tested.

#### Neutralization Protocol

Neutralization experiments were performed in THP1 cells, a human monocytic cell line that naturally expresses TLR5, and HEK293 cells transfected to stably express human TLR5. 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- $\kappa$ B-inducible ELAM-1 (E-selectin) promoter<sup>4</sup>. 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/TLR5-SEAP cells

1- Prepare a 1/10 PAb-hTLR5 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-hTLR5 dilution (5 µg/ml final).

5- Incubate 10 min at 37°C.

6- Add 10 ng/ml of flagellin.

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
293/hTLR5	293-htlr5
pUNO-hTLR5 (human gene)	puno-htlr5
Standard flagellin ( <i>S. typhimurium</i> )	tlr1-stfla
Recombinant flagellin ( <i>S. typhimurium</i> )	tlr1-flic
pNiFty-SEAP	pnifty-seap
QUANTI-Blue™	rep-qb-1

#### TECHNICAL SUPPORT

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