PRODUCT INFORMATION

Content
• 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 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-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, lipoolarabinomannan 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 mycoplasma lipopeptide1, and associates with TLR1 to recognize triacylated lipopetides2. Furthermore, pathogen recognition by TLR2 is strongly enhanced by CD143.

References

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 µg/ml) using culture medium with heat inactivated FBS.
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

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TECHNICAL SUPPORT
Toll free (US): 888-457-5873
Outside US: (+1) 858-457-5873
Europe: +33 562-71-69-39
E-mail: info@invivogen.com
Website: www.invivogen.com