

MONOCLONAL ANTIBODY

# Anti-TLR4 (CD284) (Human) mAb-FITC

Code No.	Clone	Subclass	Quantity
D077-4	HTA125	Mouse IgG2a	1 mL (50 tests)

**BACKGROUND:** Toll, a *Drosophila* receptor molecule with extracellular leucine-rich repeat (LRR), has a role in triggering innate defenses against bacteria or fungi. Toll-like receptor 4 (TLR4/CD284) is a member of TLR family, which is human homologue of Toll protein. It has extracellular LRR and an intracellular signaling domain, which is similar to the type I IL-1 receptor. TLR4 is expressed in subpopulations of cells including myeloid cells, B cells, monocytes, and endothelial cells. Recent studies have suggested that TLR4 might act as a receptor for lipopolysaccharide (LPS). TLR4 alone is not capable of sensing and signaling the presence of LPS, but another molecule MD-2, which is physically associated with TLR4, is required for LPS recognition through TLR4.

**SOURCE:** This antibody was purified from hybridoma (clone HTA125) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0 with Balb/c mouse splenocyte immunized with human TLR4 transfected cells.

**FORMULATION:** 80 µg IgG in 1 mL volume of PBS containing 1% BSA and 0.09% NaN<sub>3</sub>.

\*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

**STORAGE:** This antibody solution is stable for one year from the date of purchase when stored at 4°C.

**REACTIVITY:** This antibody reacts with human TLR4 on Flow cytometry.

**APPLICATIONS:**

Flow cytometry; 20 µL (ready for use)

\*Please refer to the data sheet (MBL code no. D077-3) for other applications.

Detailed procedure is provided in the following **PROTOCOL.**

**SPECIES CROSS REACTIVITY:**

Species	Human	Mouse	Rat
Cell	Ttransfectant	Not tested	Not tested
Reactivity on FCM	+		

**INTENDED USE:**

For Research Use Only. Not for use in diagnostic procedures.

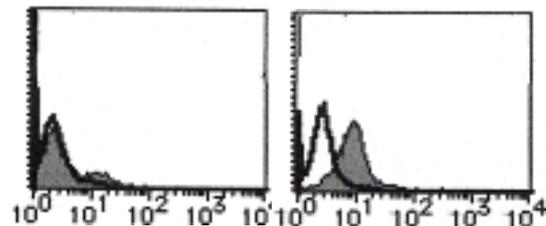
**REFERENCES:**

- 1) Suzuki, T., *et al.*, *Infect. Immun.* **72**, 1856-1865 (2004)
- 2) Uehara, A., *et al.*, *Clin Diagn Lab Immunol.* **10**, 286-292 (2003)
- 3) Tsutsumi-Ishii, Y., *et al.*, *J. Immunol.* **170**, 4226-4236 (2003)
- 4) Akashi, S., *et al.*, *Biochem. Biophys. Res. Commun.* **268**, 172-177 (2000)
- 5) Rehli, M., *et al.*, *J. Biol. Chem.* **275**, 9773-9781 (2000)
- 6) Shimazu, R., *et al.*, *J. Exp. Med.* **189**, 1777-1782 (1999)
- 7) Wright, S. D., *J. Exp. Med.* **189**, 605-609 (1999)
- 8) Medzhitov, R., *et al.*, *Nature* **388**, 394-397 (1997)
- 9) Lemaitre, B. E., *et al.*, *Cell* **86**, 973-983 (1996)

Clone HTA125 is used in reference number 1) - 4) and 6).

**RELATED PRODUCTS:**

- K0210-3 Anti-TLR1 (CD281) (Human) mAb (GD2.F4)
- K0211-3 Anti-TLR2 (CD282) (Mouse) mAb (mT2.7)
- K0212-3 Anti-TLR2 (CD282) mAb (T2.5)
- D077-3 Anti-TLR4 (CD284) (Human) mAb (HTA125)
- D077-4 Anti-TLR4 (CD284) (Human) mAb-FITC (HTA125)
- D077-5 Anti-TLR4 (CD284) (Human) mAb-PE (HTA125)
- D205-3 Anti-TLR4 (CD284) (Mouse) mAb (UT49)
- D205-4 Anti-TLR4 (CD284) (Mouse) mAb-FITC (UT49)
- D079-3 Anti-TLR4-MD-2 complex (Mouse) mAb (MTS510)
- D079-4 Anti-TLR4-MD-2 complex (Mouse) mAb-FITC (MTS510)
- D079-5 Anti-TLR4-MD-2 complex (Mouse) mAb-PE (MTS510)
- D206-3 Anti-TLR4-MD-2 complex (Mouse) mAb (UT15)
- D206-5 Anti-TLR4-MD-2 complex (Mouse) mAb-PE (UT15)
- K0213-3 Anti-TLR9 (CD289) mAb (5G5)



**Flow cytometric analysis of human TLR4 expression on transfectant (right) and parental cells (left).** Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D077-4 to the cells.

**PROTOCOL:**

**Flow cytometric analysis for floating cells**

We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

- 1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN<sub>3</sub>].
- 2) Resuspend the cells with washing buffer (5x10<sup>6</sup> cells/mL).
- 3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
- 4) Add 10 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN<sub>3</sub> to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
- 5) Add the primary antibody at the amount of suggested in the **Applications**. Mix well and incubate for 30 minutes at room temperature.
- 6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 7) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Transfectant)