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

MONOCLONAL ANTIBODY

FITC-labeled Anti-Mouse TLR4/CD284

Code No.	Clone	Subclass	Quantity	Concentration
D205-4	UT49	Mouse IgG2b	100 µL	500 µg/mL

BACKGROUND: Toll, a Drosophila receptor molecule with extracellular leucine-rich repeat (LRR), has a role in triggering innate defenses against bacteria or fungi. TLR4 (Toll-like receptor 4) 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 LPS (lipopolysaccharide). 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 culture supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0 with TLR4 knockout C57BL/6 mouse splenocyte immunized with the mouse TLR4-MD-2 transfected cells.

FORMULATION: 50 µg IgG in 100 µL volume of PBS containing 1% BSA and 0.09% NaN_3.
*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 mouse TLR4 but doesn't react with mouse MD-2 on Flow cytometry.

APPLICATION:
Flow cytometry; 10 µg/mL (final concentration)
*Please refer to the data sheet (MBL; code no. D205-3) for other applications.

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

INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

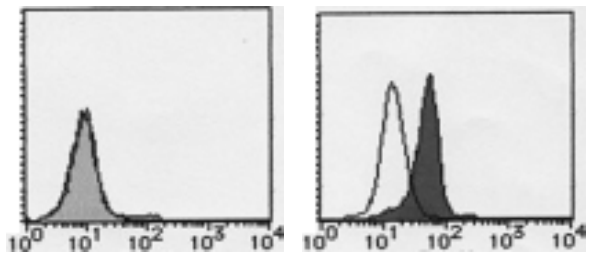
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Not Tested	Transfectant	Not Tested
Reactivity on FCM		+	

REFERENCES:
1) Tsutsumi-I, Y., *et al., J. Immunol*, **170**, 4226-4236 (2003)
2) Uehara, A., *et al., Clin. Diagn. Lab. Immunol.* **10**, 286-292 (2003)
3) Akashi, S., *et al., Biochem. Biophys. Res. Commun.* **268**, 172-177 (2000)
4) Shimazu, R., *et al., J. Exp. Med*. **189**, 1777-1782 (1999)

RELATED PRODUCTS:

D210-3	Anti-TLR1/CD281 (GD2.F4)
K0211-3	Anti-Mouse TLR2/CD282 (mT2.7)
K0212-3	Anti-Mouse TLR2/CD282 (T2.5)
D077-3	Anti-Human TLR4/CD284 (HTA125)
D077-4	FITC labeled Anti-Human TLR4/CD284 (HTA125)
D077-5	PE labeled Anti-Human TLR4/CD284 (HTA125)
D079-3	Anti-Mouse TLR4-MD-2 complex (MTS510)
D079-4	FITC labeled Anti-Mouse TLR4-MD-2 complex (MTS510)
D079-5	PE labeled anti- Mouse TLR4-MD-2 complex (MTS510)
D205-3	Anti-Mouse TLR4/CD284 (UT49)
D206-3	Anti-Mouse TLR4-MD-2 complex (MTS510)
D206-5	PE labeled anti-Mouse TLR4-MD-2 complex (MTS510)
K0213-3	Anti-Mouse TLR9/CD289 (5G5)



Flow cytometric analysis of FITC labeled Mouse TLR4 expression on mouse TLR4-MD-2 transfected Ba/F3 cells (right) and Ba/F3 parental cells (left). *Open histogram indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D205-4 to the cells.*

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL https://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

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_3].
2) Resuspend the cells with washing buffer ($5x10^6$ 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_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 20 μL of the primary antibody at the concentration of as suggest in the **APPLICATIONS** diluted in the washing buffer. 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)