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

MONOCLONAL ANTIBODY

Anti-TLR4 (CD284) (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D205-3	UT49	Mouse IgG2b	100 µL	1 mg/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 knock out C57BL/6 mouse splenocyte immunized with mouse TLR4-MD-2 transfected Ba/F3 cell.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with mouse TLR4 but doesn't react with mouse MD-2 on Flow cytometry[2].

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation; Not tested*
*It is reported that clone UT49 can be used in immunoprecipitation analysis of TLR4-transfected Ba/F3 cells[2].
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 5 µg/mL (final concentration)

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

INTENDED USE:

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SPECIES CROSS REACTIVITY:

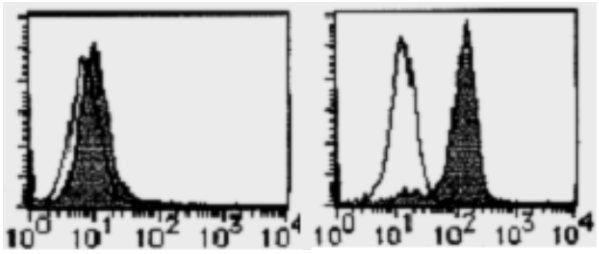
Species	Human	Mouse	Rat
Cell	Not tested	Transfectant	Not tested
Reactivity on FCM		+	

REFERENCES:

1) Kozako, T., *et al., Mol. Immunol.* **47**, 606-613 (2009)
2) Bahrun, U., *et al., Hybridoma* **26**, 393-399 (2007)
3) Tsutsumi-Ishii, Y., *et al., J. Immunol*, **170**, 4226-4236 (2003)
4) Uehara, A., *et al., Clin. Diagn. Lab. Immunol.* **10**, 286-292 (2003)
5) Akashi, S., *et al., Biochem. Biophys. Res. Commun.* **268**, 172-177 (2000)
6) Shimazu, R., *et al., J. Exp. Med*. **189**, 1777-1782 (1999)

RELATED PRODUCTS:

D205-4 Anti-TLR4 (CD284) (Mouse) mAb-FITC (UT49)
D206-3 Anti-TLR4-MD-2 complex (Mouse) mAb (UT15)
D206-5 Anti-TLR4-MD-2 complex (Mouse) mAb-PE (UT15)
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)
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)
M077-3 Mouse IgG2b (isotype control) (3D12)



Flow cytometric analysis of mouse TLR4 expression on Ba/F3 parental cells (left) and mouse TLR4-MD-2 transfected Ba/F3 cells (right). Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D205-3 to the cells.

PROTOCOLS:

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 0.09% 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.

2) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
3) Add 100 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 0.09% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 40 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) Add FITC conjugated anti-mouse IgG antibody diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
8) 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.
9) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Transfectant)