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

MONOCLONAL ANTIBODY

Anti-Mouse TLR4-MD-2 complex

Code No.	Clone	Subclass	Quantity	Concentration
D079-3	MTS510	Rat IgG2a κ	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 G agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0 with Wister rat lymph node cells immunized with mouse Ba/F3 cells expressing mouse TLR4-MD-2 complex.

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

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

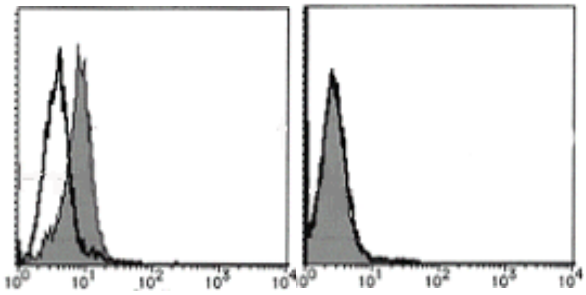
REACTIVITY: This antibody reacts with mouse TLR4-MD-2 complex on Flow cytometry. It doesn't react with mouse TLR4 alone. Clone MTS510 inhibits the TNF-α production from peritoneal macrophages by LPS stimulation*.
*It is reported in reference number 12).

APPLICATIONS:
Western blotting; Not tested
Immunoprecipitation; Not tested*
Neutralization; Not tested*
*It is reported that clone MTS510 can be used in these applications in the reference number 12).
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10-20 µg/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse		Rat
Cells	Not Tested	Ba/F3 cells expressing mouse TLR4-MD-2	Ba/F3 cells expressing mouse TLR4 alone	Not Tested
Reactivity on FCM		+	-	



Flow cytometric analysis of TLR4-MD-2 complex expression on Ba/F3 parental cells (right) or mouse TLR4 and MD-2 co-transfected Ba/F3 cells (left). *Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D079-3 to the cells.*

INTENDED USE:
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4) Saitoh, S., *et al*., *Int. Immunol*. **16**, 961-969 (2004)
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Clone MST510 is used in reference number 1) - 12).

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PROTOCOL:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps 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 20 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 40 μL of the primary antibody at the concentration 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 30 μL of 1:40 FITC conjugated anti-rat IgG (MBL; code no. IM-0827) 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.

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)
D079-4	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)
D205-4	FITC labeled anti-Mouse TLR4/CD284 (UT49)
D206-3	anti-Mouse TLR4-MD-2 complex (UT15)
D206-5	PE labeled anti-Mouse TLR4-MD-2 complex (UT15)
K0213-3	anti-Mouse TLR9/CD289 (5G5)
M081-3	Rat IgG2a Isotype control (2H3)
M081-4	FITC labeled Rat IgG2a Isotype control (2H3)
M081-5	PE labeled Rat IgG2a Isotype control (2H3)
M081-8	Agarose conjugated Rat IgG2a Isotype control (2H3)