

MONOCLONAL ANTIBODY

Anti-TLR2/CD282

Code No.	Clone	Subclass	Quantity	Concentration
K0212-3	T2.5	Mouse IgG1	100 µg	1 mg/mL

BACKGROUND: Toll, a Drosophila receptor molecule with extracellular leucine-rich repeat (LRR), has a role in triggering innate defense against bacteria or fungi. Toll-like receptor 2 (TLR2) is a member of TLR family. Members of the TLR family are components of the mammalian anti-microbial response, signaling with a domain closely related to that of IL-1 receptors. TLR2 is expressed on the surface of cells and is responsible for distinguishing different pathogens. TLR2 has been identified as a receptor that is central to innate immune response to components of Gram-negative or Gram-positive bacteria. And a co-operative effect is observed between TLR2 and TLR6. For example, TLR2/TLR6 heterodimer recognizes lipopeptide derived from mycoplasma or zymosan. TLR2 and TLR4 are pattern recognition receptors and signaling molecules in response to bacterial lipoproteins and have been implicated in innate immunity and inflammation.

SOURCE: This antibody was purified from hybridoma (clone T2.5) using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3X with TLR2 knockout mouse splenocyte immunized with the recombinant protein corresponding to the N-terminal 587 amino acid of mouse TLR2.

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 TLR2 on Flow cytometry.

APPLICATIONS:

- Western blotting; Not recommended
- Immunoprecipitation; Not recommended
- Immunohistochemistry; Not tested
- Immunocytochemistry; Not tested
- Flow cytometry; 10 µg/mL (final concentration)

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

INTENDED USE:

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	THP-1 Monocyte Granulocyte	P3U1	Not Tested
Reactivity on FCM	+	+	

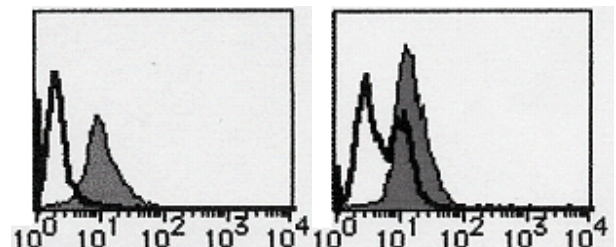
REFERENCES:

- 1) Spiller, S., *et al.*, *J. Biol. Chem.* **282**, 13190-13198 (2007)
- 2) Hise, G. A., *et al.*, *J. Immunol.* **178**, 1068-1076 (2007)
- 3) Wang, Q., *et al.*, *J. Exp. Med.* **203**, 2853-2863 (2006)
- 4) Leemans, C. J., *et al.*, *J. Clin. Invest.* **115**, 2894-2903 (2005)
- 5) Roura-Mir, C., *et al.*, *J. Immunol.* **175**, 1758-1766 (2005)
- 6) Grabcic, A., *et al.*, *J. Biol. Chem.* **279**, 48004-48012 (2004)
- 7) Meng, G., *et al.*, *J. Clin. Invest.* **113**, 1473-1481 (2004)

Clone T2.5 is used in these references.

RELATED PRODUCTS:

- D210-3 Anti-TLR1/CD281 (GD2.F4)
- K0211-3 Anti-Mouse TLR2/CD282 (mT2.7)
- 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)
- D205-4 FITC labeled 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 TLR2 (T2.5) expression on THP-1 cells (left) and P3U1 cells (right). Open histograms indicate the reaction of Isotypic control to the cells. Shaded histograms indicate the reaction of K0212-3 to the cells.

PROTOCOLS:

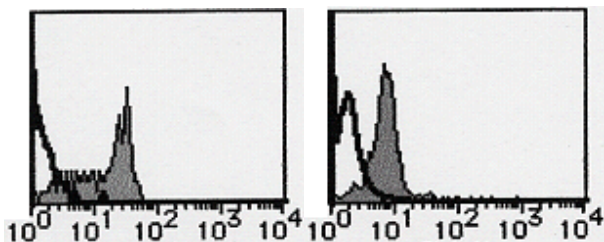
Flow cytometric analysis for floating cells

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 0.1% NaN₃].
- 2) Resuspend the cells with washing buffer (5x10⁶ 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% NaN₃ 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 30 µL of 1:40 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) 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.

- 2) Add 50 µL of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25°C).
- 3) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 4) Add 30 µL of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) diluted with washing buffer. Mix well and incubate for 15 minutes at room temperature.
- 5) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 6) Lyse with OptiLyse C (for analysis on Beckman Coulter instruments) or OptiLyse B (for analysis on BD instruments), using the procedure recommended in the respective package inserts.
- 7) Add 1 mL of H₂O to each tube and incubate for 10 minutes at room temperature.
- 8) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 9) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 10) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; THP-1, P3U1)



Flow cytometric analysis of TLR2 (T2.5) expression on human monocytes (left) and granulocytes (right). Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of K0212-3 to the cells.

Flow cytometric analysis for whole blood cells

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

- 1) Add 20 µL of anti-TLR2/CD282 monoclonal antibody (T2.5) (10 µg/mL) diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN₃] into each tube.