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

MONOCLONAL ANTIBODY

Anti-Mouse TLR9/CD289

Code No.	Clone	Subclass	Quantity	Concentration
K0213-3	5G5	Mouse IgG2a	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 9 (TLR9) 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. Recognition by innate immune cells of the pathogen associated molecular patterns (PAMPs) bacterial CpG-DNA depends on TLR9. TLR9 is dispersedly and intracellularily expressed in resting cells. Upon CpG ODN treatment, TLR9 is recruited to endosome and initiates signal transduction, which activates mitogen-activated protein kinases (MAPKs) and NF-κB through the MyD88/IRAK/TRAF6 kinases cascade.

SOURCE: This antibody was purified from culture supernatant using protein A agarose. This hybridoma (clone 5G5) was established by fusion of mouse myeloma cell P3X with C57BL/6 mouse splenocyte immunized with the recombinant human TLR9 (1-815 aa).

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 TLR9 on Flow cytometry. Reactivity of 5G5 to endogenous human TLR9 is not confirmed in our laboratory. However, it is reported that this clone reacts with human TLR9 in reference number 2).

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

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

INTENDED USE:
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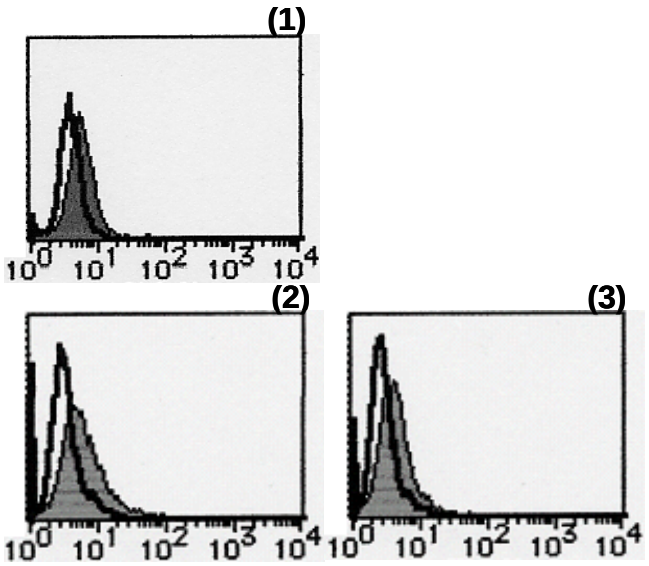
SPECIES CROSS REACTIVITY:

Species	Human		Mouse	Rat
Cells	THP-1 Monocyte Granulocyte	Transfectant	L5178Y Transfectant	Not Tested
Reactivity on FCM	-	+	+	

REFERENCES:
1) Pawar, D. R., *et al.*, *J. Am. Soc. Nephrol.* **17**, 141-149 (2006)
2) Ahmad-Nejad, P., *et al.*, *Eur. J. Immunol.* **32**, 1958-1968 (2002)

Clone 5G5 is used in these references.



Flow cytometric analysis of Mouse TLR9 expression in L5178Y cells (1), mouse TLR9 transfected CHO cells (2) and human TLR9 transfected CHO cells (3). *Open histograms indicate the reaction of Isotypic control to the cells. Shaded histograms indicate the reaction of K0213-3 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_3].

2) Add 200 μL of 4% paraformaldehyde (PFA) to the cell pellet after tapping. Mix well, then fix the cells for 30 minutes at 4°C.
3) Wash the cells 3 times with washing buffer.
4) Add 200 μL of 100 μg/mL digitonin (in PBS) to the cell pellet after tapping. Mix well, then permeablize the cells for 30 minutes at room temperature (20~25°C).
5) Wash the cells 3 times with washing buffer.
6) 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.
7) 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.
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) 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 30 minutes at room temperature.
10) 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.
11) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; L5178Y, mouse TLR9 transfectant, human TLR9 transfectant)

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)
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)