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

Anti-TLR8 (CD288) (Human) pAb

CODE No. PD047

CLONALITY Polyclonal
ISOTYPE Rabbit Ig, affinity purified
QUANTITY 100 μL

SOURCE Purified Ig from rabbit serum
REACYIVITY This antibody reacts with the full-length and cleaved C-terminal domain of TLR8 (CD288).
FORMURATION 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.

APPLICATIONS-CONFIRMED

Western blotting	1:1,000 for chemilumunescence detection system
Immunoprecipitation	Not recommended
Immunocytochemistry	1:100
Flow cytometry	1:100

SPECIES CROSS REACTIVITY on WB

Species	Human	Monkey	Mouse	Rat	Hamster
Cells	Raji, HL-60, THP-1	Not tested	Not tested	Not tested	Not tested
Reactivity	+				

Entrez Gene ID 51311 (Human)

REFERENCES

1) Ishii, N., *et al., J. Immunol.* **193**, 5118-5128 (2014) [WB, IC]
2) Itoh, H., *et al., PLoS One* **6**, e28500 (2011)

For more information, please visit our web site **http://ruo.mbl.co.jp/**

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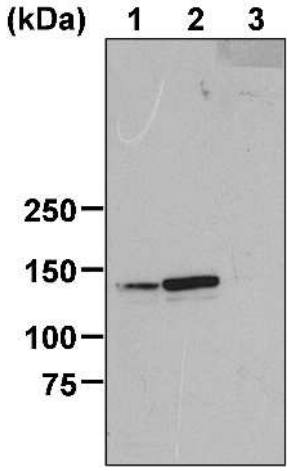
RELATED PRODUCTS

PD047	Anti-TLR8 (CD288) (Human) pAb
D077-3	Anti-TLR4 (CD284) (Human) mAb
D077-4	Anti-TLR4 (CD284) (Human) mAb-FITC
D077-5	Anti-TLR4 (CD284) (Human) mAb-PE
D079-3	Anti-TLR4-MD-2 complex (Mouse) mAb
D079-4	Anti-TLR4-MD-2 complex (Mouse) mAb-FITC
D079-5	Anti-TLR4-MD-2 complex (Mouse) mAb-PE
D205-3	Anti-TLR4 (CD284) (Mouse) mAb
D206-3	Anti-TLR4-MD-2 complex (Mouse) mAb
D206-5	Anti-TLR4-MD-2 complex (Mouse) mAb-PE
K0210-3	Anti-TLR1 (CD281) (Human) mAb
K0211-3	Anti-TLR2 (CD282) (Mouse) mAb
K0212-3	Anti-TLR2 (CD282) mAb
K0213-3	Anti-TLR9 (CD289) mAb
PM035	Normal Rabbit IgG

SDS-PAGE & Western blotting

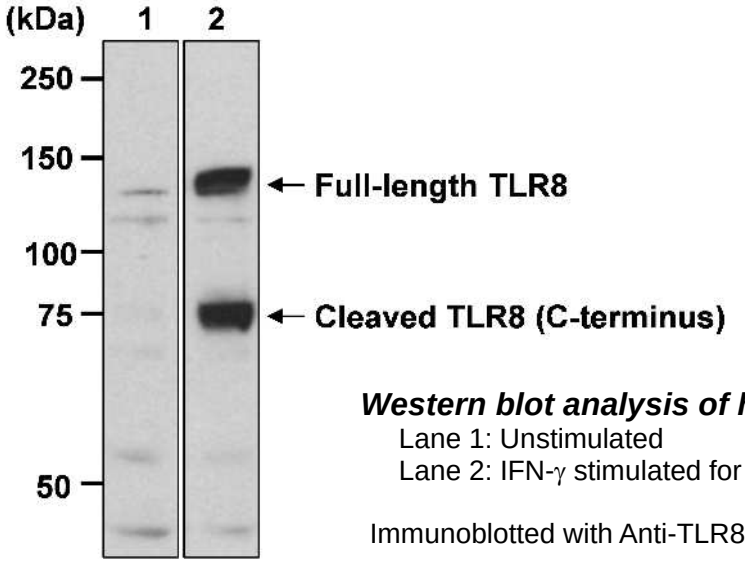
1) Wash 1 x 10^7 cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
2) Boil the samples for 3 min. and centrifuge.
3) Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. in a dry transfer system.
5) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
7) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with PBS-T (5 min. x 3 times).
9) Incubate the membrane with 1:10,000 of Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
10) Wash the membrane with PBS-T (5 min. x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
12) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; Raji, HL-60 and THP-1)



Western blot analysis of human TLR8 (CD288)
Lane 1: Raji
Lane 2: HL-60
Lane 3: HeLa

Immunoblotted with Anti-TLR8 (CD288) (Human) pAb (PD047)



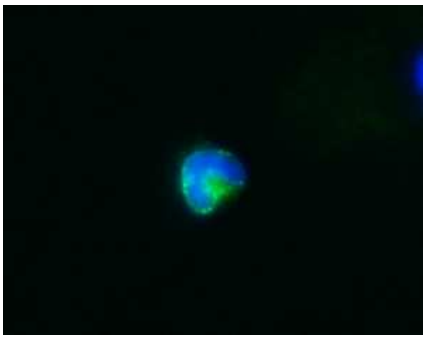
Western blot analysis of human TLR8 (CD288) from THP-1 cells
Lane 1: Unstimulated
Lane 2: IFN-γ stimulated for 3 days

Immunoblotted with Anti-TLR8 (CD288) (Human) pAb (PD047)

Immunocytochemistry

1) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde (PFA) for 30 min. at room temperature (20~25°C).
2) Wash the slide 3 times with PBS.
3) Permeabilize the cells with 0.1% Triton X-100/PBS for 10 min. at room temperature.
4) Wash the slide 3 times with PBS.
5) Tip off PBS and add the primary antibody diluted with 2% fetal calf serum (FCS)/PBS as suggested in the **APPLICATIONS** onto the cells. Incubate for 30 min. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
6) Wash the slide 3 times with PBS.
7) Tip off PBS and add 1:400 Goat Anti-rabbit IgG (H + L) Secondary Antibody, Alexa Fluor®488 conjugate (Thermo Fisher Scientific; code no. A-11008) diluted with PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
8) Visualize by reacting for 5 min. with DAPI.
9) Wash the slide 3 times with PBS. Now ready for mounting.

(Positive control for Immunocytochemistry; HL-60)



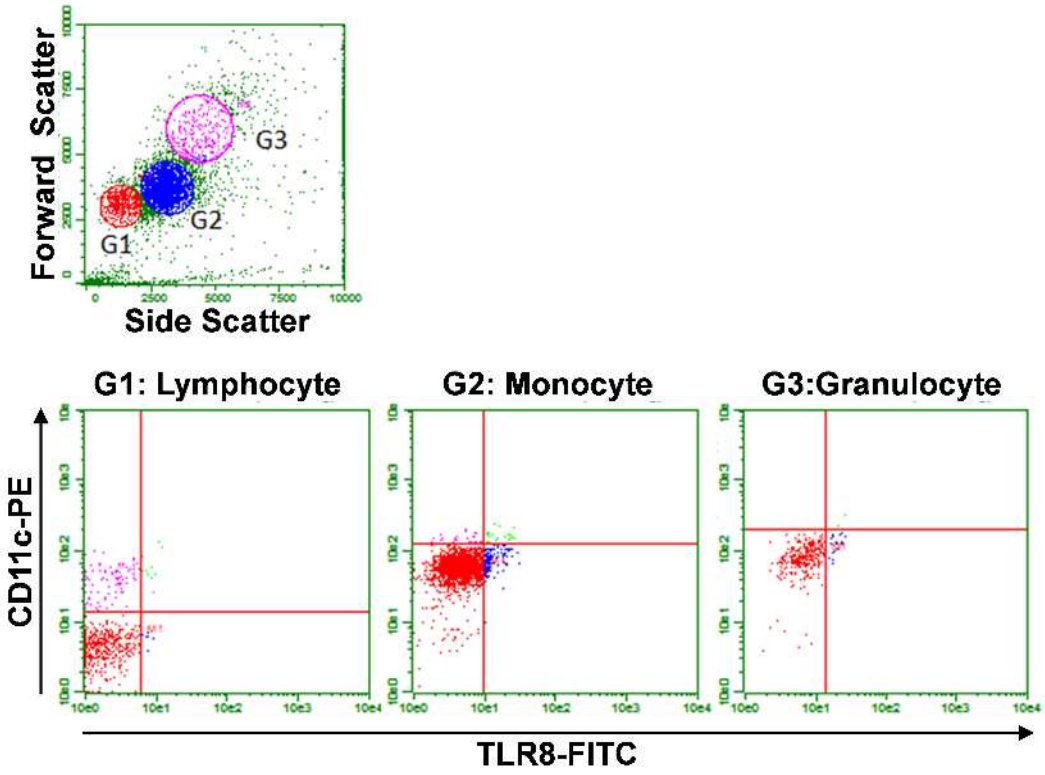
Immunocytochemical detection of human TLR8 (CD288) on HL-60 cell

Blue: DAPI
Green: Anti-TLR8 (CD288) (Human) pAb (PD047)

Flow cytometric analysis for whole blood cells

1) Dispense 100 µL of whole blood into each tube.
2) Add 100 µL of OptiLyse B (for analysis on BD instruments, Beckman Coulter; code no. IM-1400). Mix well and incubate for 10 min. at room temperature (20~25°C).
3) Add 1 mL of distilled water to each tube and incubate for 10 min. at room temperature.
4) Centrifuge at 500 x g for 1 min. at room temperature. Remove supernatant by careful aspiration.
5) Fix the cells with 4% paraformaldehyde (PFA) for 10 min. at room temperature.
6) Wash the cells 2 times with 1 mL of 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.
7) Permeabilize the cells with 0.1% Triton X-100/PBS for 10 min. at room temperature
8) Wash the cells 1 time with 1 mL of washing buffer.
9) Add 10 µL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 10 min. at room temperature.
10) Add 40 µL of primary antibody diluted washing buffer as suggested in the **APPLICATIONS**. Mix well and incubate for 30 min. at room temperature.
11) Wash the cells 1 time with 1 mL of washing buffer.
12) Add FITC conjugated anti-rabbit IgG antibody diluted with washing buffer. Mix well and incubate for 15 min. at room temperature.
13) Wash the cells 1 time with 1 mL of washing buffer.
14) Add 20 µL of PE Mouse Anti-Human CD11c (BD Biosciences; code no. 555392). Mix well and incubate for 15 min. at room temperature.
15) Wash the cells 1 time with 1 mL of washing buffer.
16) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Human PBMC)

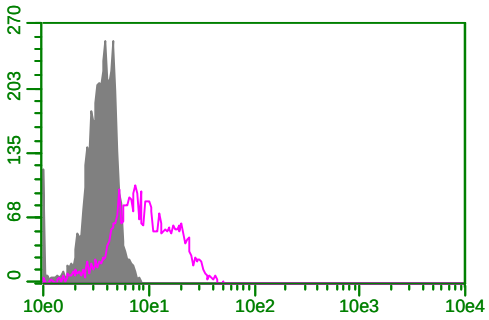


Flow cytometric analysis of TLR8 (CD288) on human PBMC
Antibody: Anti-TLR8 (CD288) (Human) pAb (PD047)

Flow cytometric analysis for floating cells

1) Wash the cells (2.4 x 10^5 cells/sample) 1 time with 1 mL of 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) Fix the cells with PBS containing 4% paraformaldehyde (PFA) for 10 min. at room temperature.
3) Wash the cells 2 times with 1 mL of washing buffer.
4) Permeabilize the cells with 0.1% Triton X-100/PBS for 10 min. at room temperature
5) Wash the cells 1 time with 1 mL of washing buffer.
6) Add 10 µL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 10 min. at room temperature.
7) Add 40 µL of primary antibody diluted washing buffer as suggested in the **APPLICATIONS**. Mix well and incubate for 30 min. at room temperature.
8) Wash the cells 1 time with 1 mL of washing buffer.
9) Add FITC conjugated anti-rabbit IgG antibody diluted with washing buffer. Mix well and incubate for 15 min. at room temperature.
10) Wash the cells 1 time with 1 mL of washing buffer.
11) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; HL-60)



Flow cytometric analysis of human TLR8 (CD288) on HL-60 cells
Open: Anti-TLR8 (CD288) (Human) pAb (PD047)
Closed: Normal Rabbit IgG (PM035)