

# Anti-CD279 (PD-1) (Human) mAb

## (Functional Grade)

<b>CODE No.</b>	D133-3M2
<b>CLONALITY</b>	Monoclonal
<b>CLONE</b>	J105
<b>ISOTYPE</b>	Mouse IgG1 $\kappa$
<b>QUANTITY</b>	100 $\mu$ L, 1 mg/mL
<b>SOURCE</b>	Purified IgG from hybridoma supernatant
<b>IMMUNOGEN</b>	Human PD-1 Fc fusion protein
<b>FORMURATION</b>	PBS. Azide free, 0.22 $\mu$ m sterile-filtered Endotoxin level is < 0.5 EU/mg antibody, as determined by the LAL assay.
<b>STORAGE</b>	This antibody solution is stable for one year from the date of purchase when stored at -20°C.

### APPLICATION-CONFIRMED

Flow cytometry 5  $\mu$ g/mL

### SPECIES CROSS REACTIVITY on FCM

Species	Human	Mouse	Rat	Hamster
Cells	PHA-stimulated PBMC, transfectant	Not tested	Not tested	Not tested
Reactivity	+			

**Entrez Gene ID** 5133 (Human)

**REFERENCES**  
1) Kanai, T., *et al.*, *J. Immunol.* **171**, 4156-4163 (2003)  
2) Iwai, Y., *et al.*, *Immunol. Lett.* **83**, 215-220 (2002)

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## **RELATED PRODUCTS**

### Functional grade antibodies

D133-3M2 Anti-CD279 (PD-1) (Human) mAb FG (J105)  
D292-3M2 Anti-Mincle (Mouse) mAb FG (4A9)  
D266-3M2 Anti-Mincle (Mouse) mAb FG (1B6)  
M187-3 Anti-IL-33 (Mouse) mAb FG (1F11)  
M188-3 Anti-IL-33 (Mouse) mAb FG (2C7)  
M075-3M2 Mouse IgG1 (isotype control) FG (2E12)  
M076-3M2 Mouse IgG2a (isotype control) FG (6H3)  
M079-3M2 Mouse IgM (isotype control) FG (7E10)  
M080-3M2 Rat IgG1 (isotype control) FG (1H5)

### Purified antibodies

D133-3 Anti-CD279 (PD-1) (Human) mAb (J105)  
D133-5 Anti-CD279 (PD-1) (Human) mAb-PE (J105)  
D132-3 Anti-CD279 (PD-1) (Human) mAb (J110)  
D132-4 Anti-CD279 (PD-1) (Human) mAb-FITC (J110)  
D132-5 Anti-CD279 (PD-1) (Human) mAb-PE (J110)  
D092-3 Anti-CD274 (PD-L1) (Human) mAb (MIH3)  
D092-6 Anti-CD274 (PD-L1) (Human) mAb-Biotin (MIH3)  
D230-3 Anti-CD274 (PD-L1) (Human) mAb (27A2)  
D230-5 Anti-CD274 (PD-L1) (Human) mAb-PE (27A2)

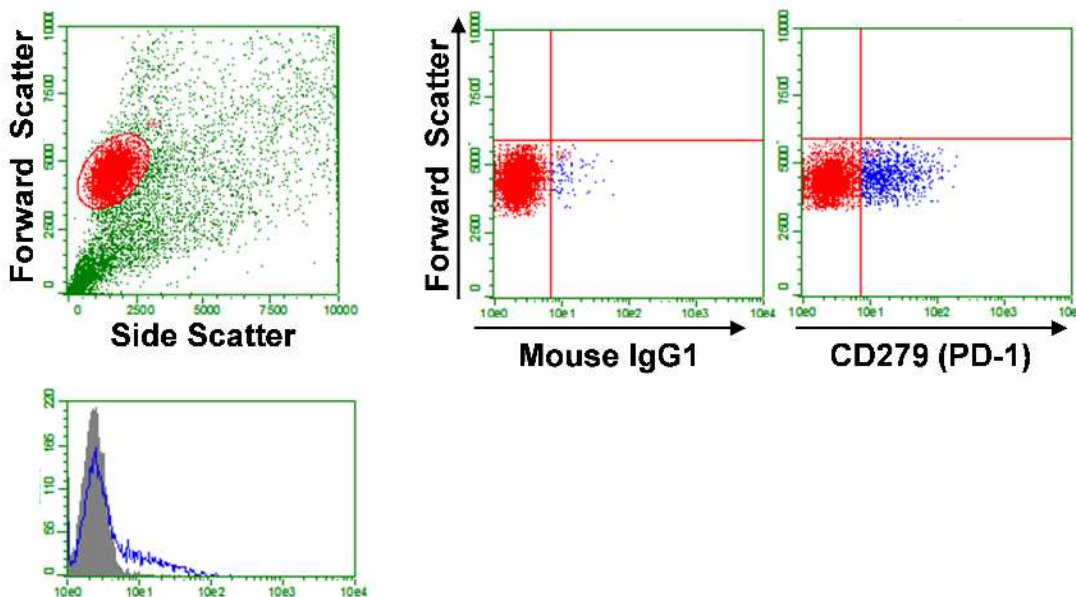
Other related antibodies and kits are also available.

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**Flow cytometric analysis for human PBMCs**

- 1) Stimulate human PBMCs with 5 µg/mL of PHA for 3 days.
- 2) Wash the cells ( $4 \times 10^5$  cells/sample) 1 time with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN<sub>3</sub>]. \*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.
- 3) 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.
- 4) Add 40 µL of the primary antibody at the concentration as suggested in the **APPLICATION** diluted with washing buffer. Mix well and incubate for 30 min. at room temperature.
- 5) Wash the cells 1 time with 1 mL of washing buffer.
- 6) Add 40 µL of 1:400 Goat anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor® 488 conjugate (Thermo Fisher Scientific; code no. A-11001) diluted with washing buffer. Mix well and incubate for 15 min. at room temperature.
- 7) Wash the cells 1 time with 1 mL of washing buffer.
- 8) 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.
- 9) Add 1 mL of distilled water to each tube and incubate for 10 min. at room temperature.
- 10) Centrifuge at 500 x g for 1 min. at room temperature. Remove supernatant by careful aspiration.
- 11) Wash the cells 1 time with 1 mL of washing buffer.
- 12) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; PHA-stimulated human PBMC)

**Flow cytometric analysis of CD279 (PD-1) on PHA-stimulated human PBMC**

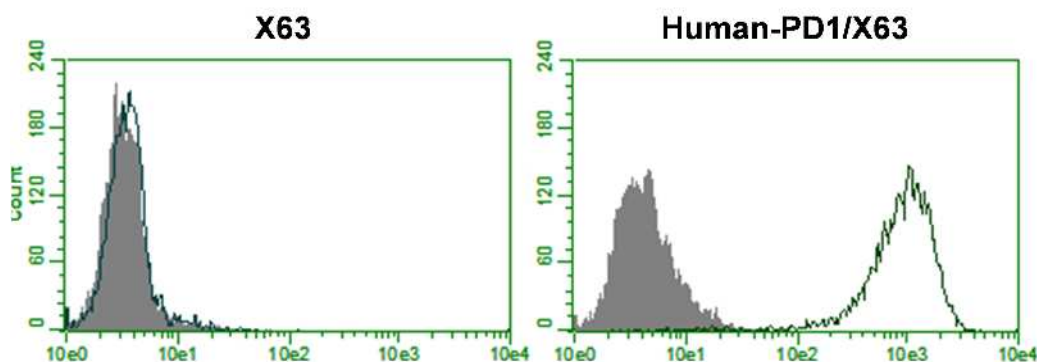
Open: Anti-CD279 (PD-1) (Human) mAb (D133-3M2)

Closed: Mouse IgG1 (isotype control) (M075-3)

**Flow cytometric analysis for floating cells**

- 1) Wash the cells ( $3 \times 10^5$  cells/sample) 1 time with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
- 2) Add 10  $\mu$ L 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.
- 3) Add 20  $\mu$ L of the primary antibody at the concentration as suggested in the **APPLICATION** diluted with washing buffer. Mix well and incubate for 15 min. at room temperature.
- 4) Wash the cells 1 time with 1 mL of washing buffer.
- 5) Add 40  $\mu$ L of 1:400 Goat anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor<sup>®</sup> 488 conjugate (Thermo Fisher Scientific; code no. A-11001) diluted with washing buffer. Mix well and incubate for 15 min. at room temperature.
- 6) Wash the cells 1 time with 1 mL of washing buffer.
- 7) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer.

(Positive control for flow cytometry; Transfectant)

**Flow cytometric analysis of human CD279 (PD-1) on transfectant**

Open: Anti-CD279 (PD-1) (Human) mAb (D133-3M2)

Closed: Mouse IgG1 (isotype control) (M075-3)