

MONOCLONAL ANTIBODY

Anti-CD226 (DNAM-1) (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D172-3	TX25	Mouse IgG1 κ	100 μ L	1 mg/mL

BACKGROUND: CD226/DNAM-1 (DNAM accessory molecule-1) is a 65 kDa type I transmembrane glycoprotein expressed on the majority of T cells, NK cells, monocytes, and a subset of B-cells. A soluble form (50 kDa) of CD226 is present in normal serum and the supernatant of cultured activated T cells. Ligand binding to CD226 results in phosphorylation of Ser329 and Tyr322, suggesting a role in signal transduction. CD226 acts as an adhesion molecule involved in the binding of certain tumor cells to CTL and NK cells and mediates their cytotoxicity. CD226 also stimulates TNF- α and IFN- γ secretion by alloantigen-specific T cells.

SOURCE: This antibody was purified from hybridoma (clone TX25) supernatant using protein G agarose.

IMMUNOGEN: Recombinant human CD226 (DNAM-1) protein.

FORMULATION: 100 μ g IgG in 100 μ L 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 human CD226 (DNAM-1) antigen on Flow cytometry.

APPLICATIONS:

- Western blotting; Not tested
- Immunoprecipitation; Not tested
- Immunohistochemistry; Not tested
- Immunocytochemistry; Not tested
- Flow cytometry; 5-10 μ g/mL (final concentration)

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

INTENDED USE:

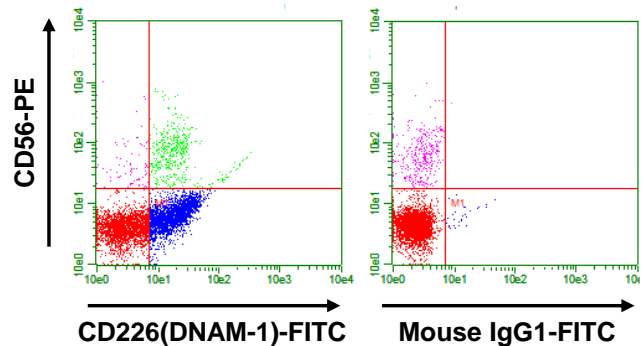
For Research Use Only. Not for use in diagnostic procedures.

REFERENCE:

- 1) Shibuya, K., *et al.*, *J. Exp. Med.* **198**, 1829-1839 (2003)

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	PBMC	Not tested	Not tested
Reactivity on FCM	+		



Flow cytometric analysis of human CD226 (DNAM-1) expression (left) and isotypic control (right) on human PBMC. The staining intensity of D172-3 is shown in the horizontal axis with human CD56 staining on the vertical axis.

PROTOCOL:

Flow cytometric analysis for whole blood cells

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

- 1) Add 20 μ L of CD56-PE (Beckman Coulter; code no. A07788) into each tube.
- 2) Add 100 μ L of whole blood into each tube. Mix well, and incubate for 20 minutes at room temperature (20~25°C).
- 3) Add 1 mL of the washing buffer (PBS containing 2% fetal calf serum) followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 4) Add 20 μ L of anti-CD226 (DNAM1) (Human) mAb (D172-3) at the concentration as suggested in the **APPLICATIONS** diluted with the washing buffer. Mix well and incubate for 20 minutes at room temperature.
- 5) 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.
- 6) Add 20 μ L of 1:100 anti-Mouse IgG-FITC (Beckman Coulter; code no. IM-0819) diluted with the washing

buffer. Mix well and incubate for 20 minutes at room temperature.

- 7) Add 1 mL of deionized water to each tube and incubate for 10 minutes at room temperature.
- 8) 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.
- 9) Add 1 mL of deionized water to each tube and incubate for 10 minutes at room temperature.
- 10) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 11) 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.
- 12) Resuspend the cells with 500 µL of the washing buffer and analyze a flow cytometer.

(Positive control for Flow cytometry; Human PBMC)

RELATED PRODUCTS:

- D172-4 Anti-CD226 (DNAM-1) (Human) mAb-FITC (TX25)
- D146-3 Anti-Nectin-1 (Mouse) mAb (48-12)
- D083-3 Anti-CD112 (Nectin-2) (Mouse) mAb (502-57)
- D175-3 Anti-CD112 (Human) mAb (TX31)
- D175-4 Anti-CD112 (Human) mAb-FITC (TX31)
- D174-3 Anti-CD155 (Human) mAb (TX21)
- D174-4 Anti-CD155 (Human) mAb-FITC (TX21)
- D174-5 Anti-CD155 (Human) mAb-PE (TX21)