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



## MONOCLONAL ANTIBODY

# **CD112**

Code No. Clone Subclass Quantity Concentration D175-3 TX31 Rat IgG2a 100 µg 1 mg/mL

**BACKGROUND:** CD112, also known as Poliovirus Receptor Related 2 (PRR2), is a transmembrane glycoprotein that functions as the receptor for alpha-herpes virus and pseudorabies virus entry into the cell. CD112 has been shown to be identical to Nectin-2, an adhesion protein localized to adherens junctions. CD112 is widely expressed in neuronal, endothelial, and in particular, epithelial cells, where it is found at the intercellular junctions of adjacent cells. Additionally CD112 is detected on hematopoietic progenitors expressing CD34, where it plays a role in the regulation of hematopoietic/endothelial cell interactions. At least two isoforms of CD112 mRNA have been identified.

**SOURCE:** This antibody was purified from hybridoma (clone TX31) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell Sp2/0 with Wister rat lymphocyte immunized with the recombinant human CD112.

**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 human CD112 antigen on Flow cytometry.

# **APPLICATIONS:**

<u>Western blotting</u>; Not tested <u>Immunoprecipitation</u>; Not tested <u>Immunohistochemistry</u>; Not tested <u>Immunocytochemistry</u>; Not tested

Flow cytometry; 5-10 µg/mL (final concentration)

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

## **SPECIES CROSS REACTIVITY:**

Species	Human	Mouse	Rat
Cell	KG-1	Not Tested	Not Tested
Reactivity on FCM	+		

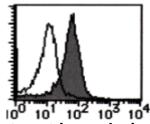
#### **INTENDED USE:**

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#### **REFERENCES:**

- 1) Tahara-Hanaoka, S., et al., Blood 107, 1491-1496 (2006)
- 2) Tahara-Hanaoka, S., et al., Int. Immunol. 16, 533-538 (2004)
- 3) Bottino, C., et al., J. Exp. Med. 198, 557-567 (2003)

Clone TX31 is used in reference number 1) and 2).



Flow cytometric analysis of CD112 expression on KG-1. Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D175-3 to the cells.

# **PROTOCOL:**

# Flow cytometric analysis for floating cells

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

- 1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN<sub>3</sub>].
- 2) Resuspend the cells with washing buffer (5x10<sup>6</sup> cells/mL).
- 3) Add 50  $\mu$ L 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 20  $\mu L$  of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
- 5) Add 30  $\mu$ L of the primary antibody at the concentration 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.

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- 7) Add 30  $\mu$ L of 1:200 PE conjugated anti-rat IgG (MBL; code no. IM-1623) 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  $\mu L$  of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; KG-1)

#### **RELATED PRODUCTS:**

- D172-3 CD226 (TX25)
- D172-4 FITC labeled CD226 (TX25)
- D174-3 CD155 (TX21)
- D174-4 FITC labeled CD155 (TX21)
- D174-5 PE labeled CD155 (TX21)
- D175-4 FITC labeled CD112 (TX31)
- D146-3 Mouse CD111/Nectin-1 (48-12)
- D083-3 Mouse CD112/Nectin-2 (502-57)
- D084-3 Mouse CD113/Nectin-3 (103-A1)