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

MONOCLONAL ANTIBODY

CD229/Ly9

Code No.	Clone	Subclass	Quantity	Concentration
K0038-3	HLy-9.1.25	Mouse IgG1 κ	100 μg	1 mg/mL

BACKGROUND: CD229, also known as Ly9/SLAMF3, is a 120 kDa cell surface glycoprotein. It belongs to the member of CD150 family, a group of structurally related leukocyte cell surface receptors of the IgSF. Expression of CD229 is restricted to mature T- and B-lymphocytes and thymocytes. The cytoplasmic domain of CD229 contains two unique tyrosine-based motifs (T-I/V-Y-x-x-V/I), which are binding sites for SLIM-associated protein (SAP). In the immunodeficiency, X-linked lymphoproliferative disease, CD150 family of SAP-binding cell surface receptors may relate to regulation of the immune system.

SOURCE: This antibody was purified from hybridoma (clone Hly-9.1.25) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell NS-1 with Balb/c mouse splenocyte immunized with the mouse pre-B-cell line 300.19, stably transfected with the full-length human Ly9 cDNA.

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 CD229 antigen on Flow cytometry.

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation; Not tested*
*It is reported that this monoclonal antibody can be used in Immunoprecipitation in the reference number 1).
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10 μg/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	peripheral blood lymphocyte	Not Tested	Not Tested
Reactivity on FCM	+		

INTENDED USE:
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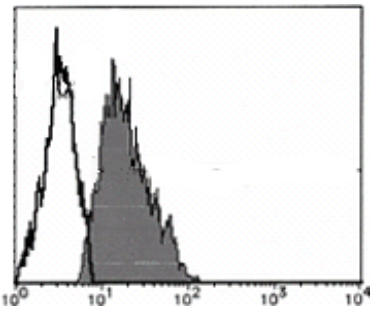
REFERENCES:

1) Martín, M., *et al., J. Immunol.* **174**, 5977-5986 (2005)
2) Del Valle, J. M., *et al., J. Biol. Chem.* **278**, 17430-17437 (2003)
3) De la Fuente, M. A., *et al., Blood* **97**, 3513-3520 (2001)

Clone HLy-9.1.25 is used in these references.

RELATED PRODUCTS:

K0038-4	CD229/Ly9-FITC (HLy-9.1.25)
M075-3	Mouse IgG1 isotype control (2E12)
M075-4	Mouse IgG1 isotype control-FITC (2E12)



Flow cytometric analysis of CD229 expression on human peripheral blood lymphocyte. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of K0038-3 to the cells.*

PROTOCOLS:

Flow cytometric analysis for whole blood cells

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

1) Add 50 μL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3] into each tube.
2) Add 50 μL of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25 °C).
3) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
4) Add 30 μL of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. 238) diluted with washing buffer. Mix

well and incubate for 15 minutes at room temperature.
5) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
6) 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.
7) Add 1 mL of H_2O to each tube and incubate for 10 minutes at room temperature.
8) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
10) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; lymphocyte)

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_3].
2) Resuspend the cells with washing buffer (5 x 10^6 cells/mL).
3) Add 100 μL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25^{o}C). Remove supernatant by careful aspiration.
4) Add 20 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 5 minutes at room temperature.
5) Add 40 μL of the primary antibody at the concentration as suggested 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.
7) Add 30 μL of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. 238) 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 μL of the washing buffer and analyze by a flow cytometer.