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Not for use in diagnostic procedures.

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MONOCLONAL ANTIBODY

Anti-CD160 (Human) mAb

Code No.	Clone	Subclass	Quantity
K0122-1	CL1-R2	Mouse IgG1	100 µL

BACKGROUND: CD160, also known as BY55 antigen, is a GPI-anchored cell surface receptor found on a small subset of T-effector cells that have cell killing activity. CD160, which has a broad specificity for MHC class Ia and Ib molecules, behaves as a co-receptor upon T cell activation. Similarly, binding of CD160 to HLA-C has been shown to activate cytotoxic function in NK cells. CD160 monoclonal antibody has potential therapeutic use as an anti-inflammatory or tolerance-promoting drug to eliminate self-reactive T-effector cells.

SOURCE: This antibody was concentrated from hybridoma (clone CL1-R2) supernatant. This hybridoma was established by fusion of mouse myeloma cell NS-1 with Balb/c mouse splenocyte immunized with CD160 transfected Jurkat cells.

FORMULATION: 100 µL volume of PBS with preservative (0.09% NaN_3). This antibody was concentrated from hybridoma supernatant and dialyzed against PBS.

*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.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody reacts with CD160 antigen on Flow cytometry. Clone CL1-R2 inhibits the binding of CD160 receptor to its MHC class I ligands.

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation; Not tested*
*It is reported that clone CL1-R2 can be used in Immunoprecipitation in the reference number 8).
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 1:100 (final concentration)
Function; Not tested*
*It is reported that this antibody can be used as an agonist[1] or a blocking antibody[2), 3)].

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

INTENDED USE:
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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	NK92, lymphocyte	Not tested	Not tested
Reactivity on FCM	+		

REFERENCES:

1) Zuo, J., *et al*., *J. Transl. Med*. **13**, 188 (2015) [Function]
2) El-Far, M., *et al*., *J. Transl. Med*. **12**, 217 (2014) [Function]
3) Kojima, R., *et al*., *J. Mol. Biol.* **413**,762-772 (2011) [Function]
4) Giustiniani, J., *et al., J. Immunol*. **178**, 1293-1300 (2007)
5) Fons, P., *et al., Blood* **108**, 2608-2615 (2006)
6) Barakonyi, A., *et al., J. Immunol.* **173**, 5349-5354 (2004)
7) Bouteiller, L.P., *et al., PNAS* **99**, 16963-16968 (2002)
8) Nikolova, M., *et al., Int. Immunol*. **14**, 445-451 (2002)
9) Agrawal, S., *et al*., *J. Immunol*. **162**, 1223-1226 (1999)

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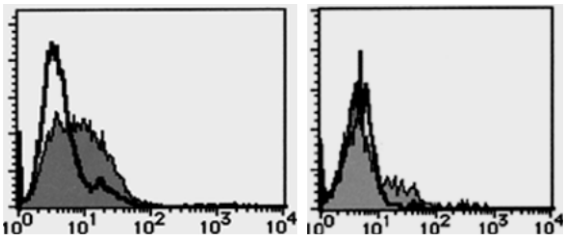

Flow cytometric analysis of CD160 expression on NK92 cells (left) and Lymphocytes (right). *Open histograms indicate the reaction of Isotypic control to the cells. Shaded histograms indicate the reaction of K0122-1 to the cells.*

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

Flow cytometric analysis for floating cells
We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

1) Wash the cells 3 times with 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) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
3) Add 50 µL 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 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 of 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.
7) Add FITC conjugated anti-mouse IgG antibody 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.

(Positive controls for Flow cytometry; NK92)

Flow cytometric analysis for whole blood cells

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

1) Add 10 µL of the 1:10 Anti-CD160 (Human) mAb (K0122-1) diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3] into each tube.
2) Add 100 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 FITC conjugated anti-mouse IgG antibody 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.