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

MBL
A JSR Life Sciences Company

MONOCLONAL ANTIBODY

Anti-HLA-A2 (Human) mAb-PE

Code No.	Clone	Subclass	Quantity
K0186-5	BB7.2	Mouse IgG2b	1 mL (50 tests)

BACKGROUND: Human leukocyte antigen-A2 (HLA-A2) is a class I molecule of the human major histocompatibility complex (MHC). It is a heterodimer composed of a 43 kDa α subunit that is non-covalently associated with the 12 kDa β2-microglobulin protein. HLA-A2, like other class I molecules, binds peptides from proteins degraded in the cytoplasm and plays a role in antigen presentation and interaction with $CD8^+$ T cells. HLA-A2 is the most common HLA-A allele in the Caucasian and American Indian populations (50% and ~30%, respectively) and A2 restricted T cell epitopes have been studied widely.

SOURCE: This antibody was purified from hybridoma (clone BB7.2) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell NS-1 with Balb/c mouse splenocyte immunized with papain solubilized HLA-A2.

FORMULATION: 50 tests in 1 mL volume of PBS containing 1% BSA and 0.1% ProClin 150.

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

REACTIVITY: This antibody reacts with human HLA-A2 on Flow cytometry.

APPLICATION:
Flow cytometry; 20 µL (Ready for use)
*Please refer to the data sheet (MBL; code no. K0186-3) for other applications.

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	T2	Not tested	Not tested
Reactivity on FCM	+		

INTENDED USE:
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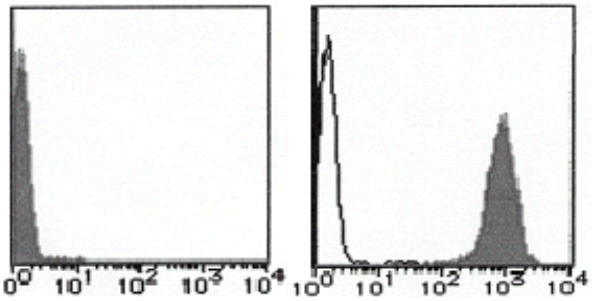
REFERENCES:
1) Shen, H., *et al*., *Cancer Immunol. Immunother.* **62,** 393-403 (2013) [FCM]
2) Kozako, T., *et al*., *J. Immunol*. **177**, 5718-5726 (2006)
3) Yamano, Y., *et al., J. Exp. Med.* **199**, 1367-1377 (2004)
4) Rodolfo, M., *et al., Cancer Res*. **63**, 6948-6955 (2003)
5) Smith, M. E. F., *et al., PNAS.* **86**, 5557-5561 (1989)
6) Parham, P., *et al., Human Immunology* **3**, 277-299 (1981)

Clone BB7.2 is used in these references.

As clone BB7.2 is really famous all over the world, a lot of researches have been reported. These references are a part of such reports.



Flow cytometric analysis of HLA-A2 expression on T2 cells (right) and Jurkat cells (left). *Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of K0186-5 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 (5 x 10^6 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 20 µL of the primary antibody. 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) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; T2)

Flow cytometric analysis for whole blood cells

We usually use Fisher tubes or equivalents as reaction tubes for all step described below.
1) Add 20 µL of the primary antibody 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) 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.
5) Add 1 mL of H_2O to each tube and incubate for 10 minutes at room temperature.
6) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
7) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
8) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

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