

MONOCLONAL ANTIBODY

# Anti-HLA-A24 (Human) mAb-PE

Code No.	Clone	Subclass	Quantity
K0209-5	22E1	Mouse IgG2b	1mL (50 tests)

**BACKGROUND:** HLA (human leukocyte antigen)-A24 is a class I MHC antigen. HLA-A24 is the most frequent HLA class I molecule in Asian populations, present in approximately ~70% of the Japanese population. HLA-A24 is also found in approximately 35% of the Indian population and 19% of Caucasians. HLA antigens may play a role in genetic susceptibility to disease.

**SOURCE:** This antibody was purified from hybridoma (clone 22E1) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell Sp2/0 with C57BL/6 Tg mouse splenocyte immunized with the recombinant human HLA-A24.

**FORMULATION:** 50 tests in 1 mL volume of PBS containing 1% BSA and 0.09% NaN<sub>3</sub>.

\*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 HLA-A24 on Flow cytometry.

**Note:** It was reported that this clone 22E1 cross-reacted to HLA-B27 and some indeterminate HLA. Although HLA-B27 population is so small in Japanese, about 20% of tested population in our laboratories reacted to this antibody as false-positive. To ensure your experiment, you should confirm HLA genotyping.

## APPLICATION:

Flow cytometry: 20 µL (Ready for use)

\*Please refer to the data sheet (MBL; code no. K0209-3) for other applications.

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

## SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	LCL721	Not tested	Not tested
Reactivity on FCM	+		

## INTENDED USE:

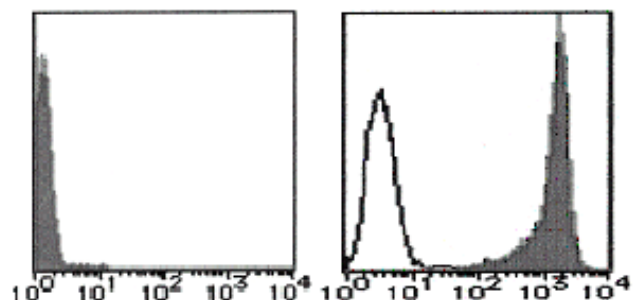
For Research Use Only. Not for use in diagnostic procedures.

## REFERENCES:

- 1) Najima, Y., *et al.*, *Blood* **127**,722-734 (2016)
- 2) Mimura, K., *et al.*, *Cancer Sci.* **105**, 1236-1244 (2014)
- 3) Shen, H., *et al.*, *Cancer Immunol. Immunother.* **62**, 393-403 (2013)
- 4) Lutz, C. T., *et al.*, *J. Immunol.* **153**, 4099-4110 (1994)
- 5) Tahara, T., *et al.*, *Immunogenetics* **32**, 351-360 (1990)

Clone 22E1 is used in these references.

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



**Flow cytometric analysis of HLA-A24 expression on LCL721 cells (right) and Jurkat Cells (left).** Open histogram indicate the traction of isotypic control to the cells. Shaded histograms indicate the reaction of K0209-5 to the cells.

## PROTOCOLS:

### 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 0.09% NaN<sub>3</sub>].  
\*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<sup>6</sup> 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  $\mu$ L 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  $\mu$ L of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; LCL721)

### **Flow cytometric analysis for whole blood cells**

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

- 1) Add 20  $\mu$ L of the primary antibody into each tube.
- 2) Add 50  $\mu$ L 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 [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN<sub>3</sub>] followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.  
\*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.
- 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<sub>2</sub>O 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  $\mu$ L of the washing buffer and analyze by a flow cytometer.

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