

MONOCLONAL ANTIBODY

Anti-HLA-A24 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
K0208-3	17A10	Mouse IgG2b	100 μ L	1 mg/mL

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 17A10) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell Sp2/0 with C57BL/6Tg mouse splenocyte immunized with human recombinant HLA-A24.

FORMULATION: 100 μ g IgG in 100 μ L 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 HLA-A24 on Flow cytometry.

Note: It was reported that this clone 17A10 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.

APPLICATIONS:

- Western blotting; Not tested
- Immunoprecipitation; Not tested
- Immunohistochemistry; Not tested
- Immunocytochemistry; Not tested
- Flow cytometry; 10 μ g/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

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

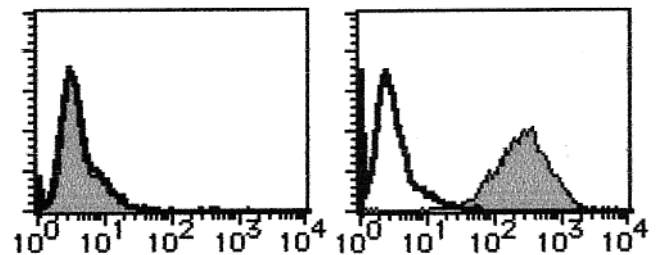
INTENDED USE:

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

REFERENCES:

- 1) Kobayashi, E., *et al.*, *Nat. Med.* **19**, 1542-1546 (2013)
- 2) Kozako, T., *et al.*, *J. Immunol.* **177**, 5718-5726 (2006)
- 3) Lutz, C. T., *et al.*, *J. Immunol.* **153**, 4099-4110 (1994)
- 4) Tahara, T., *et al.*, *Immunogenetics* **32**, 351-360 (1990)

Clone 17A10 is used in these references.



Flow cytometric analysis of HLA-A24 expression on Jurkat cells (Left) and LCL721 cells (Right). Open histogram indicates the reaction of Isotypic control to the cells. Shaded histograms indicate the reaction of K0208-3 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 0.1% NaN_3].
- 2) Resuspend the cells with washing buffer (5×10^6 cells/mL).
- 3) Add 50 μ L of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature ($20 \sim 25^{\circ}\text{C}$). Remove supernatant by careful aspiration.
- 4) Add 20 μ 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 10 minutes at room temperature.
- 5) Add 20 μ L of the primary antibody at the concentration of as suggested in the **APPLICATIONS** diluted with the washing buffer. Mix well and incubate for 15 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 40 µL of 1:100 Anti-IgG (Mouse) pAb-FITC (MBL; code no. IM-0819) 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 control for Flow cytometry; LCL721)

K0209-5	Anti-HLA-A24 (Human) mAb-PE (22E1)
D226-3	Anti-HLA class I (HLA-A,B,C) (Human) mAb (EMR8-5)
K0126-3	Anti-HLA-E (Human) mAb (MEM-E/02)
K0215-3	Anti-HLA-E (Human) mAb (4D12)
K0125-3	Anti-HLA-G (Human) mAb (MEM-G/1)
K0216-3	Anti-HLA-G (Human) mAb (87G)
K0019-1	Anti-HLA-DR (Human) mAb (LN-3)
M077-3	Mouse IgG2b (isotype control) (3D12)

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 of as suggested in the **APPLICATIONS** diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN₃] 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 40 µL of 1:100 Anti-IgG (Mouse) pAb-FITC (MBL; code no. IM-0819) 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 H₂O 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.

RELATED PRODUCTS:

K0186-3	Anti-HLA-A2 (Human) mAb (BB7.2)
K0186-4	Anti-HLA-A2 (Human) mAb-FITC (BB7.2)
K0186-5	Anti-HLA-A2 (Human) mAb-PE (BB7.2)
K0208-3	Anti-HLA-A24 (Human) mAb (17A10)
K0208-4	Anti-HLA-A24 (Human) mAb-FITC (17A10)
K0208-5	Anti-HLA-A24 (Human) mAb-PE (17A10)
K0208-A48	Anti-HLA-A24 (Human) mAb -Alexa Fluor [®] 488 (17A10)
K0208-A64	Anti-HLA-A24 (Human) mAb -Alexa Fluor [®] 647 (17A10)
K0209-3	Anti-HLA-A24 (Human) mAb (22E1)
K0209-4	Anti-HLA-A24 (Human) mAb-FITC (22E1)