

MONOCLONAL ANTIBODY

# Anti-HLA-A24

Code No.	Clone	Subclass	Quantity	Concentration
K0209-3	22E1	Mouse IgG2b	100 µL	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 22E1) 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 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 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.

**APPLICATIONS:**

- Western blotting; Not tested
- Immunoprecipitation; Not tested
- 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	LCL721	Not Tested	Not Tested
Reactivity on FCM	+		

**INTENDED USE:**

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

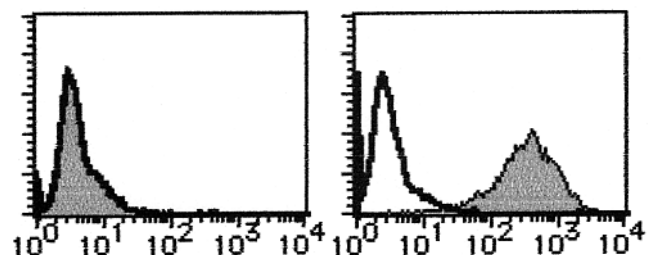
**RELATED PRODUCTS:**

- K0186-3 anti-HLA-A2 (BB7.2)
- K0186-4 FITC labeled anti-HLA-A2 (BB7.2)
- K0186-4 PE labeled anti-HLA-A2 (BB7.2)
- K0208-3 anti-HLA-A24 (17A10)
- K0208-4 FITC labeled anti-HLA-A24 (17A10)
- K0208-5 PE labeled anti-HLA-A24 (17A10)
- K0208-A48 Alexa Fluor® 488 labeled anti-HLA-A24 (17A10)
- K0208-A64 Alexa Fluor® 647 labeled anti-HLA-A24 (17A10)
- K0209-3 anti-HLA-A24 (22E1)
- K0209-4 FITC labeled anti-HLA-A24 (22E1)
- K0209-5 PE labeled anti-HLA-A24 (22E1)
- D226-3 anti-HLA-class I (HLA-A, B, C) (EMR8-5)
- K0126-3 anti-HLA-E (MEM-E/02)
- K0215-3 anti-HLA-E (4D12)
- K0125-3 anti-HLA-G (MEM-G/1)
- K0216-3 anti-HLA-G (87G)
- K0019-1 anti-HLA-DR (LN-3)
- M077-3 Mouse IgG2b isotype control (3D12)

**REFERENCES:**

- Lutz, C. T., *et al.*, *J. Immunol.* **153**, 4099-4110 (1994)
- Tahara, T., *et al.*, *Immunogenetics* **32**, 351-360 (1990)

Clone 22E1 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 K0209-3 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<sub>3</sub>].
- 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 10 minutes at room temperature.
- 5) Add 20 µL 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 FITC conjugated anti-mouse IgG (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)

### 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% NaN<sub>3</sub>] 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 FITC conjugated anti-mouse IgG (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 H<sub>2</sub>O 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.