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

MONOCLONAL ANTIBODY

# Anti-HLA-A24 (Human) mAb-Alexa Fluor™ 647

| Code No. | Clone | Subclass | Quantity | Concentration |
|---|---|---|---|---|
| K0208-A64 | 17A10 | 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 17A10) 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:** 100 µg IgG in 100 µL volume of PBS containing 1% BSA 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.

**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 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.

**APPLICATION:**
Flow cytometry; 10 µg/mL (final concentration)
*Please refer to the data sheet (MBL, code no. K0208-3) for other applications.

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

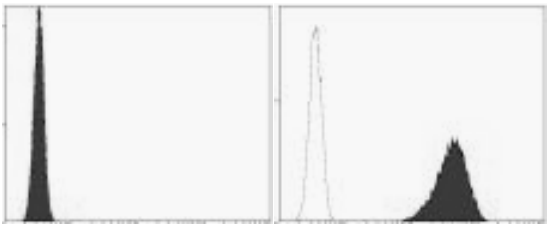
**SPECIES CROSS REACTIVITY:**

| 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.

**RELATED PRODUCTS:**
Please visit our website at https://ruo.mbl.co.jp/.

**REFERENCES:**
1) Kato, T., *et al.*, *Oncotarget* **9**, 11009-11019 (2018)
2) Ichikawa, A., *et al.*, *Oncol. Lett.* **13**, 4611-4618 (2017)
3) Harada, N., *et al.*, *J. Immunol.* **198**, 516-527 (2017)
4) Kozako, T., *et al.*, *J. Immunol.* **177**, 5718-5726 (2006)
5) Lutz, C. T., *et al.*, *J. Immunol*. **153**, 4099-4110 (1994)
6) Tahara, T., *et al.*, *Immunogenetics* **32**, 351-360 (1990)



***Flow cytometric analysis of HLA-A24 expression on Jurkat (left) and LCL721 (right).*** *Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of K0208-A64 to the cells.*

Clone 17A10 is used in these references.

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 steps described below.

1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% $NaN_3$].
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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 10 minutes at room temperature.
5) Add 20 μL of the primary antibody at the concentration of as suggested in the **APPLICATION** 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) 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 Fisher 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 **APPLICATION** diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% $NaN_3$] 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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