

MONOCLONAL ANTIBODY

Anti-HLA-E (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
K0215-3	4D12	Mouse IgG1	100 μ L	1 mg/mL

BACKGROUND: HLA-E (human leucocyte antigen-E) is a conserved class I major histocompatibility molecule. It binds to the leader peptide derived from the polymorphic classical MHC molecules HLA-A, HLA-B and HLA-C. This peptide binding stabilizes the HLA-E protein and allows it to migrate to the cell surface. HLA-E then interacts with CD94/NKG2A receptors on natural killer cells. This interaction inhibits natural killer cell-mediated lysis of cells displaying HLA-E. In virally infected or tumor cells, down-regulation of HLA-A, HLA-B and HLA-C production prevents stabilization of HLA-E by the leader peptide. Under these circumstances, HLA-E is degraded before it reaches the cell surface and the cell is then vulnerable to lysis by natural killer cells.

SOURCE: This antibody was purified from hybridoma (clone 4D12) supernatant using protein A agarose. This antibody was established by immunization of HLA-B27 transgenic mice with HLA-E protein.

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-E on Flow cytometry.

APPLICATIONS:

Western blotting; Not tested

Immunoprecipitation; Not tested

Immunohistochemistry; Not tested*

* It is reported that this monoclonal antibody can be used in immunohistochemistry on frozen section in the reference number 1).

Immunocytochemistry; Not tested

Flow cytometry; 10 μ g/mL (final concentration)

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

INTENDED USE:

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

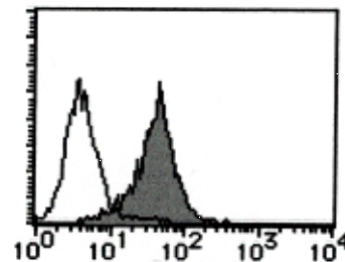
SPECIES CROSS REACTIVITY:

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

REFERENCES:

- 1) Cui, C. H., *et al.*, *Hum. Mol. Genet.* **20**, 235-244 (2011) [IHC]
- 2) Nguyen, S., *et al.*, *Bone Marrow Transplant.* **43**, 693-699 (2009) [FCM]
- 3) Tsukamoto, N., *et al.*, *Clin. Cancer Res.* **15**, 5733-5743 (2009) [FCM]
- 4) Ishitani, A., *et al.*, *J. Immunol.* **171**, 1376-1384 (2003)
- 5) Lee, N., *et al.*, *J. Immunol.* **160**, 4951-4960 (1998)

Clone 4D12 is used in reference number 1)-4).



Flow cytometric analysis of HLA-E expression on LCL721 cells. Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of K0215-3 to the cells.

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

PROTOCOL:

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% NaN₃].
*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 (5x10⁶ 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 (20~25 $^{\circ}$ 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 5 minutes at room temperature.

- 5) Add the primary antibody diluted with the washing buffer as suggested in the **APPLICATIONS**. 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) Add FITC conjugated anti-mouse IgG antibody 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 μ L of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; LCL721)

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