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

MONOCLONAL ANTIBODY

Anti-HLA-E (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
K0215-3	4D12	Mouse IgG1	100 µL	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 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-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 µg/mL (final concentration)

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

INTENDED USE:

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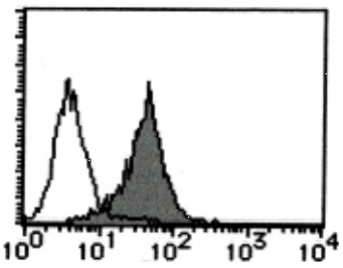
SPECIES CROSS REACTIVITY:

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% 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.
2) Resuspend the cells with washing buffer ($5x10^6$ 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

4) (20~25°C). Remove supernatant by careful aspiration.
5) 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.
6) Add the primary antibody diluted with the washing buffer as suggested in the **APPLICATIONS**. Mix well and incubate for 30 minutes at room temperature.
7) 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.
8) Add FITC conjugated anti-mouse IgG antibody diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
9) 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.
10) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; LCL721)

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