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

MONOCLONAL ANTIBODY

Anti-HLA-E

Code No.	Clone	Subclass	Quantity	Concentration
K0126-3	MEM-E/02	Mouse IgG1	100 µg	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 mouse ascites fluid using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell Sp2/0-Ag14 with Balb/c mouse splenocyte immunized with the recombinant human HLA-E.

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 the HLA-E denatured heavy chain (43 kDa) on Western blotting but does not recognize native HLA-E molecule.

APPLICATIONS:

Western blotting; 1-5 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not recommended
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested*
*Clone 4D12 (MBL; code no. K0215-3) is useful.

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

INTENDED USE:

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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	placenta		
Reactivity on W.B.	+	Not tested	Not tested

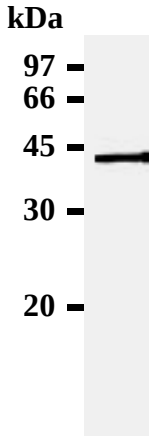
REFERENCES:

1) Coupel, S., *et al*., *Blood* **109**, 2806-2814 (2007)
2) Menier, C., *et al.*, *Hum. Immunol*. **64**, 315-26 (2003)
3) Marin, R., *et al.*, *Immunogenetics* **54**, 767-75 (2003)

Clone MEM-E/02 is used in reference number 1) - 2).

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)
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)
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)



Western blot analysis of HLA-E expression in human placenta extracts using K0126-3.

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PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; human placenta)