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MBL
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MONOCLONAL ANTIBODY

Anti-HLA class I (HLA-A,B,C) (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D367-3	EMR8-5	Mouse IgG1 κ	100 µL	1 mg/mL

BACKGROUND: Human major histocompatibility complex (MHC) class I antigen-epitope peptide complex associated with β_2-microglobulin are expressed by all human nucleated cells. It plays an important role in cell-mediated immune responses. Abnormalities in MHC class I antigen surface expression are frequently found in malignancies and infectious diseases. They are often associated with reduced recognition by MHC class I antigen-restricted, tumor or virus-associated antigen-specific cytotoxic T lymphocytes and disease progression. The EMR8-5 monoclonal antibody reacts with a non-polymorphic epitope of human MHC class I antigens, HLA-A, -B, and -C.

SOURCE: This antibody was purified from hybridoma (clone EMR8-5) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell NS-1 with Balb/c mouse splenocyte immunized with recombinant HLA-A*24:02 extracellular domain.

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 class I (HLA-A, B, C) on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL
Immunoprecipitation; Not tested
Immunohistochemistry; Can be used*
*We recommend using clone EMR8-5.1 for this application. Please refer to the data sheets (MBL, code no. D370-3H).
Immunocytochemistry; Not tested
Flow Cytometry; 1 µg/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	HL60, Jurkat, HeLa, HPB-ALL	Not tested	PC12
Reactivity on WB	+		-

INTENDED USE:

For research use only. Not for clinical diagnosis.

REFERENCES:

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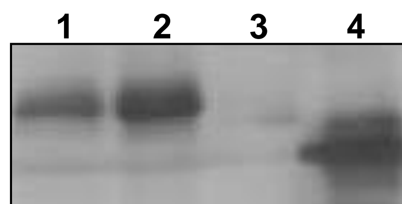
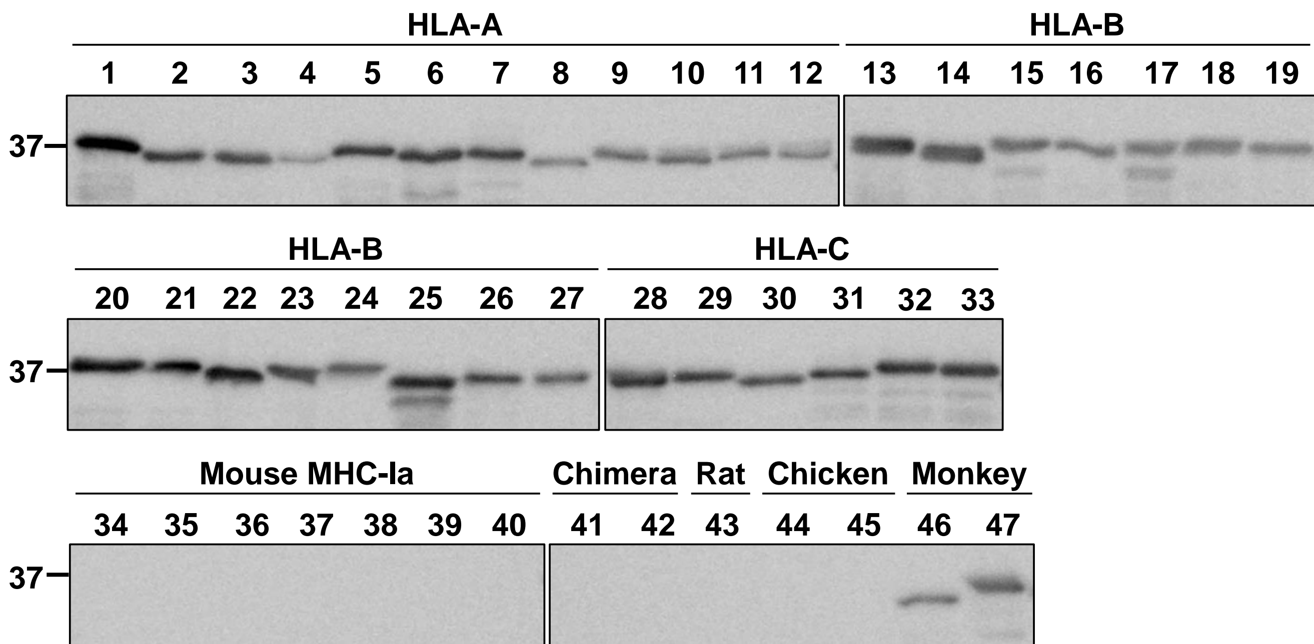
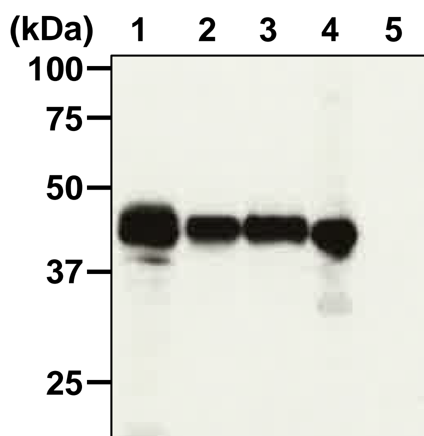
Clone EMR8-5 is used in these references.



Western blotting analysis of HLA class I expression in oral cavity cancer cell line OSC20 (1), OSC20-A*24:02 (HLA-A*24:02 transfectant) (2), K562 (3) and recombinant HLA-A*24:02 (4) using D367-3.
Data were kindly provided by Dr. Toshihiko Torigoe (The Department of Pathology, Sapporo Medical University School of Medicine).



Western blotting analysis of D367-3 reactivity using recombinant of MHC class I heavy chains extracellular domain.
1: A*01:01 2: A*02:01 3: A*02:06 4: A*02:07 5: A*03:01 6: A*11:01 7: A*23:01 8: A*24:02 (immunogen) 9: A*26:01 10: A*29:02 11: A*31:01 12: A*33:03 13: B*07:02 14: B*08:01 15: B*15:01 16: B*15:02 17: B*35:01 18: B*35:05 19: B*40:01 20: B*40:02 21: B*40:06 22: B*42:01 23: B*44:02 24: B*44:03 25: B*51:01 26: B*52:01 27: B*54:01 28: Cw*01:02 29: Cw*03:03 30: Cw*03:04 31: Cw*08:01 32: Cw*12:02 33: Cw*15:02 34: H-2K^{b} 35: H-2K^{d} 36: H-2D^{b} 37: H-2D^{d} 38: H-2D^{k} 39: H-2L^{d} 40: H-2K^{k} 41: A2K^{b} 42: A24K^{b} 43: RT1.AI 44: BF2*1201 45: BF2*1501 46: Mamu-A*90120-4 47: Mamu-A*90120-5



Western blotting analysis of HLA class I expression in HL60 (1), Jurkat (2), HeLa (3), HPB-ALL (4) and PC12 (5) using D367-3.

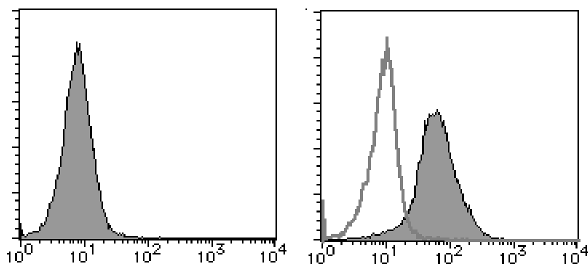
PROTOCOLS:

SDS-PAGE & Western blotting

1) Wash the cells 3 times with PBS and suspend with 1 mL of volume of Laemmli's sample buffer, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C.
3) Boil the samples for 2 minutes and centrifuge. Load 10 μL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
4) 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% methanol). See the manufacture's manual for precise transfer procedure.
5) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)

7) Wash the membrane with PBS (5 minutes x 4).
8) Incubate the membrane with the 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL, code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
9) Wash the membrane with PBS (5 minutes x 6).
10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
11) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
12) Expose to an X-ray film in a dark room for 3 minutes.
13) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Recombinant, HL60, Jurkat, HPB-ALL and HeLa)

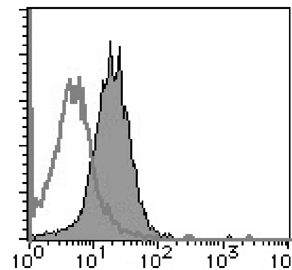


Flow cytometric analysis of HLA class I expression on K562 cells (left) and Raji cells (right). *Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D367-3 to the cells.*

Flow cytometric analysis for floating cells

1) Wash the cells 3 times with PBS.
2) Add 200 µL of 4% formaldehyde/PBS to the cell pellet after tapping. Mix well, and then fix the cells with for 10 minutes at 4°C.
3) Wash the cells with incubation buffer [PBS containing 0.5% BSA].
4) Add 10 µL of Clear back (MBL, code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at 4°C.
5) Add 100 µL of primary antibody diluted with incubation buffer as suggested in the **APPLICATIONS**. Mix well and incubate for 1 hour at 4°C. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
6) Add 1 mL of the incubation buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
7) Add 100 µL of 1:250 Anti-mouse IgG (H+L), F(ab')2 Fragment (PE conjugate) (CST, code no. 8887) diluted with incubation buffer. Mix well and incubate for 30 minutes at 4°C.
8) Add 1 mL of the incubation 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 incubation buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Raji)



Flow cytometric analysis of HLA class I expression on PBMCs. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D367-3 to the cells.*

Flow cytometric analysis for whole blood cells

1) Collect blood by venipuncture into a blood collection tube containing a salt of EDTA.
2) Add 100 µL of whole blood into a new tube. (Adjust the cell number to approximately 5×10^6 cells/mL)
3) Add 100 µL of OptiLyse® B Lysing Solution (Beckman Coulter, code no. IM1400, for analysis on BD instruments). Immediately mix well by vortex and incubate for 10 minutes at room temperature.
4) Add 1 mL of deionized water. Mix well by vortex and incubate for 10 minutes at room temperature.
5) Wash the cells with incubation buffer [PBS containing 0.5% BSA].
6) Add 10 µL of Clear back (MBL, code no. MTG-001) after tapping. Mix well and incubate for 5 minutes at 4°C.
7) Add 100 µL of primary antibody diluted with incubation buffer as suggested in the **APPLICATIONS**. Mix well and incubate for 1 hour at 4°C. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
8) Add 1 mL of incubation buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Add 100 µL of 1:250 Anti-mouse IgG (H+L), F(ab')2 Fragment (PE conjugate) (CST, code no. 8887) diluted with incubation buffer. Mix well and incubate for 30 minutes at 4°C.
10) Add 1 mL of incubation buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
11) Resuspend the cells with 500 µL of incubation buffer and analyze by a flow cytometer.

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