

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

APPLICATIONS:

Western blotting; 1 μ g/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 μ g/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	+		-

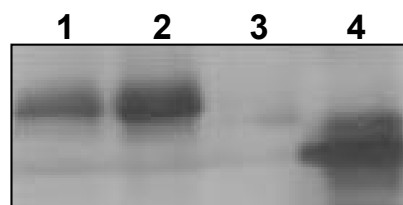
INTENDED USE:

For research use only. Not for clinical diagnosis.

REFERENCES:

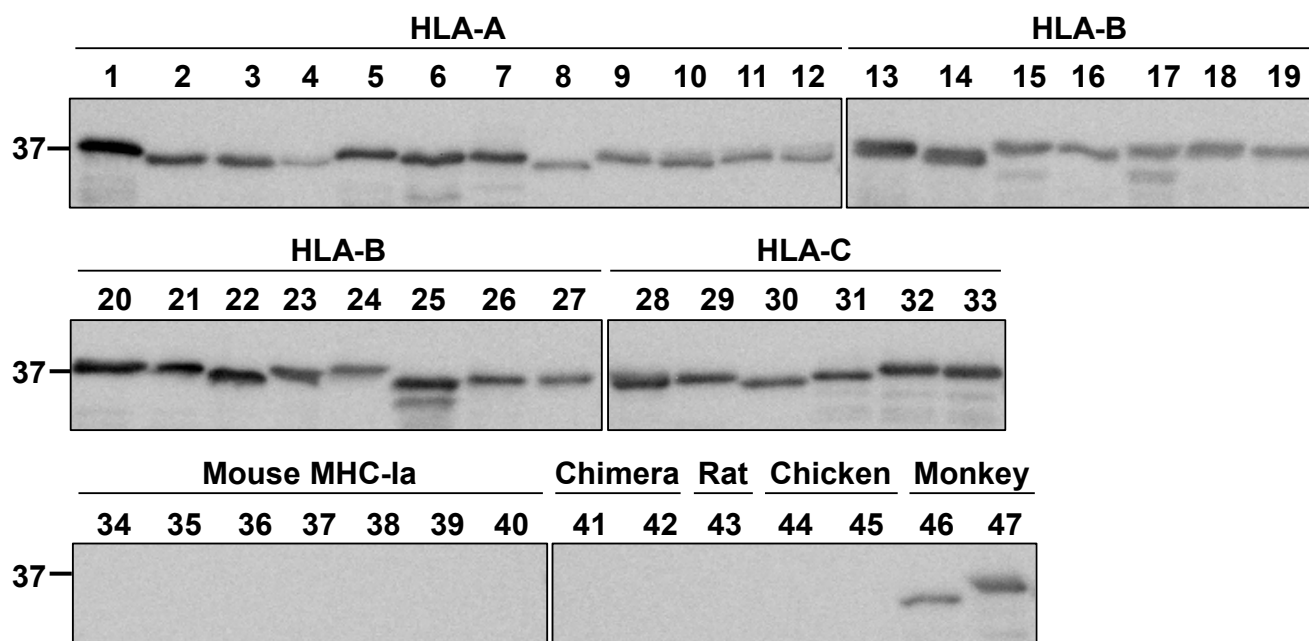
- 1) Imai, D., *et al.*, *Cancer Med.* **6**, 1614-1626 (2017)
- 2) Peng, W., *et al.*, *Cancer Discov.* **6**, 202-216 (2016)
- 3) Freeman, B. T., *et al.*, *Stem cells Transl. Med.* **4**, 685-694 (2015)
- 4) Umemoto, Y., *et al.*, *J. Gastroenterol.* **50**, 65-75 (2015)
- 5) Paulson, K. G., *et al.*, *Cancer Immunol. Res.* **2**, 1071-1079 (2014)
- 6) Wick, D. A., *et al.*, *Clin. Cancer Res.* **20**, 1125-1134 (2014)
- 7) Fujii, H., *et al.*, *Int. J. Cancer* **134**, 2393-402 (2014)
- 8) del Campo, A. B., *et al.*, *Int. J. Cancer* **134**, 102-113 (2014)
- 9) Torigoe, T., *et al.*, *Pathol. Int.* **62**, 303-308 (2012)
- 10) Halama, N., *et al.*, *Clin. Cancer Res.* **17**, 678-689 (2011)
- 11) Li, F., *et al.*, *Hum. Immunol.* **72**, 1150-1159 (2011)
- 12) Zhang, X., *et al.*, *Sci. Signal.* **3**, ra85 (2010)
- 13) Rasku, M. A., *et al.*, *J. Transl. Med.* **6**, 12 (2008)
- 14) Komori, H., *et al.*, *Clin. Cancer Res.* **12**, 2689-2699 (2006)
- 15) Kitamura, H., *et al.*, *Urology* **67**, 955-959 (2006)

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.

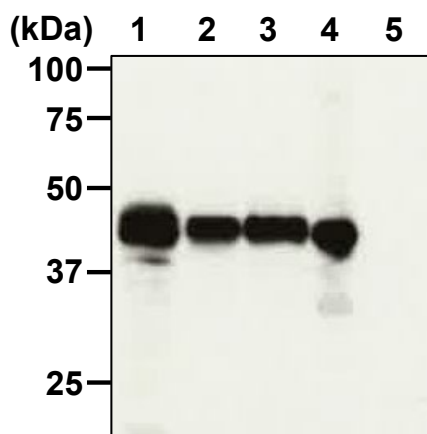
- | | | | | | |
|-----------------------|------------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| 1: A*01:01 | 2: A*02:01 | 3: A*02:06 | 4: A*02:07 | 5: A*03:01 | 6: A*11:01 |
| 7: A*23:01 | 8: A*24:02 (immunogen) | 9: A*26:01 | 10: A*29:02 | 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 | | | | | |

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

PROTOCOLS:

SDS-PAGE & Western blotting

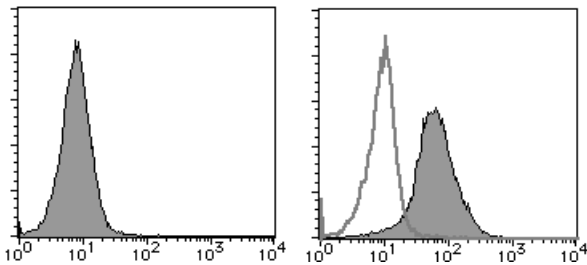
- 1) Wash the cells 3 times with PBS and suspend with 1 mL of volume of Laemmli's sample buffer, then sonicate briefly (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C.
- 3) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
- 4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² 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 10% 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.)



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

- 7) Wash the membrane with PBS-T (5 minutes x 3 times).
- 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-T (5 minutes x 3 times).
- 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)



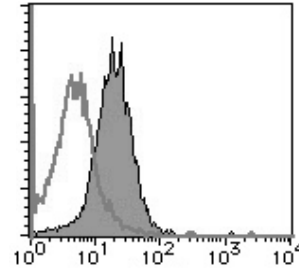
Flow cytometric analysis of HLA class I expression on K562 cells (left) and Raji cells (right). Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D367-3 to the cells.

Flow cytometric analysis for floating cells

- 1) Wash the cells 3 times with PBS.
- 2) Add 200 μ L 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 μ L 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 μ L 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 μ L 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 μ L 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 μ L of whole blood into a new tube. (Adjust the cell number to approximately 5×10^6 cells/mL)
- 3) Add 100 μ L 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 μ L of Clear back (MBL, code no. MTG-001) after tapping. Mix well and incubate for 5 minutes at 4°C.
- 7) Add 100 μ L 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 μ L 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 μ L of incubation buffer and analyze by a flow cytometer.

RELATED PRODUCTS:

Please visit our website at <https://ruo.mbl.co.jp/>.