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Not for use in diagnostic procedures.

MBL
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MONOCLONAL ANTIBODY

Anti-MICA (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
K0217-3	AMO1	Mouse IgG1 κ	100 µL	1 mg/mL

BACKGROUND: MICA and MICB (Major Histocompatibility Complex class I Chain-related gene A and gene B) bind to the activating immunoreceptor NKG2D. NKG2D is expressed on NK (Natural Killer) cells, NKT cells, γδT cells and $CD8^+$αβT cells. Recognition of MICA and MICB by NKG2D is involved in tumor surveillance, immune responses to viral infections and autoimmune diseases. MICA and MICB are transmembrane glycoproteins that are distantly related to the MIC proteins, and they possess three extra-cellular Ig-like domains. And thus, MICA and MICB are closely related but are functionally indistinguishable. MICA and MICB molecules are highly glycosylated, and are detected as a smear band ranging from 65-75 kDa. It is reported that MICA and MICB are highly expressed in variant tumor cells, whereas normal cells express little. Tumor cells have been shown to shed and release MIC molecules from the cell surface. Therefore determination of soluble MIC (sMIC) levels provides valuable information for cancer staging, and sMIC in serum seems to be an indicator for systemic manifestation of malignancy rather than for local tumor extent.

SOURCE: This antibody was purified from hybridoma (clone AMO1) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3x63Ag8.653 with Balb/c mouse splenocyte immunized with the MICA*01, MICA*04 and MICB*02 transfected P815 cells.

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 MICA on Flow cytometry and ELISA. The epitope was mapped to the helical surfaces of the MICα1α2 platform domain.

APPLICATIONS:
Western blotting; Not recommended
Immunoprecipitation; Not recommended
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10 µg/mL (final concentration)
ELISA; 1 µg/mL (for capture antibody)

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	293T, Jurkat, HeLa, DLD-1	Not tested	Not tested
Reactivity on FCM	+		

INTENDED USE:
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REFERENCES:
1) Spreu, J., *et al., J. Immunol.* **177**, 3143-3149 (2006)
2) Boissel, N., *et al., J. Immunol.* **176**, 5108-5116 (2006)
3) Armeanu, S., *et al., Cancer Res.* **65**, 6321-6329 (2005)
4) Welte, S. A., *et al., Eur. J. Immunol.* **33**, 194-203 (2003)
5) Salih, H. R., *et al., Blood* **102**, 1389-1396 (2003)
6) Salih, H. R., *et al., J. Immunol.* **169**, 4098-4102 (2002)

Clone AMO1 is used in these references.

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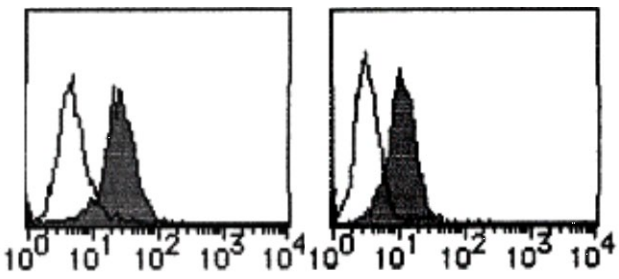
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:
Flow cytometric analysis for floating cells



Flow cytometric analysis of MICA expression on 293T cells (left) and Jurkat cells (right). *Open histograms indicates the reaction of isotypic control to the cells. Shaded histograms indicates the reaction of K0217-3 to the 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 0.1% NaN_3].
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 (20~25°C). Remove supernatant by careful aspiration.
4) 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.
5) Add 40 µL of the primary antibody at the concentration of as suggest in the **APPLICATIONS** diluted in the washing buffer. 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 30 µL of 1:100 Anti-IgG (Mouse) pAb-FITC (MBL; code no. 238) 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 µL of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; 293T, Jurkat, HeLa)

ELISA

1) Distribute 100 µL/well of Anti-MICA (Human) mAb (AMO1) (1 µg/mL) diluted with PBS to each well.
2) Incubate it overnight at 4°C.
3) Add 100 µL/well of 15% BSA/PBS.
4) Incubate it for 1 hour at 37°C.
5) Wash the plates 4 times with PBS-T [0.05% Tween-20 in PBS].
6) Distribute 100 µL/well of the samples or the recombinant MICA standard (0~15 ng/mL, American Research Products, Inc.; code no. 12-4415) diluted with 7.5% BSA/PBS to each well.
7) Incubate it for 2 hours at 37°C.
8) Wash the plates 4 times with PBS-T.
9) Distribute 100 µL/well of Anti-MICA/B (Human) mAb (BAMO3) (1 µg/mL, MBL; code no. K0218-3) to each well.
10) Incubate it for 2 hours at 37°C.
11) Wash the plates 4 times with PBS-T.
12) Distribute 100 µL/well of the 1:5,000 Anti-IgG2a (Mouse) mAb-HRP (MBL; code no. 732412) diluted with 3.75% BSA/PBS to each well.
13) Incubate it for 1 hour at 37°C.
14) Wash the plates 6 times with PBS-T.
15) Distribute 100 µL/well of the tetra-methylbenzidine (TMB) containing solution (Moss Substrates and Conjugates Inc.; code no. TMBE-1000).
16) Incubate it for 5~60 minutes. The condition for reaction may vary.
17) Distribute 100 µL/well of 1 M H_2SO_4 to each well and stop enzyme reaction.
18) After gentle mixing, determine the absorbance at 450 nm of each well by a spectrophotometer.