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

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MONOCLONAL ANTIBODY

Anti-MICA/B (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
K0219-3	BAMO1	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 BAMO1) 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/B on Western blotting and Flow cytometry. The epitope was mapped to the helical surfaces of the MICα1α2 platform domain.

INTENDED USE:
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APPLICATIONS:
Western blotting; 1 µg/mL
Immunoprecipitation; Not recommended
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10 µg/mL (final concentration)
ELISA; Not tested*
*It is reported that this monoclonal antibody can be used in ELISA in the reference number 5).

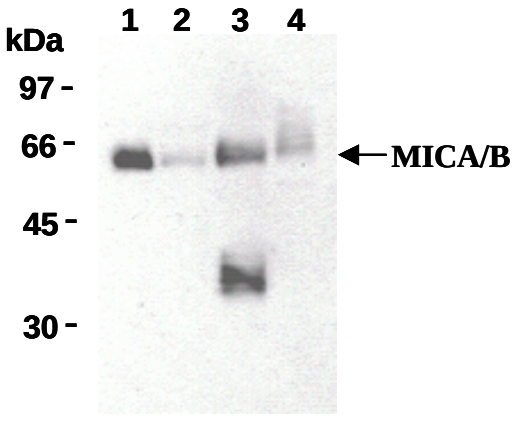
Detailed procedure is provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

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

REFERENCES:
1) Spreu, J., *et al., J. Immunol.* **177**, 3143-3149 (2006)
2) Armeanu, S., *et al., Cancer Res.* **65**, 6321-6329 (2005)
3) Wu, J. D., *et al., J. Clin. Invest.* **114**, 560-568 (2004)
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 BAMO1 is used in these references.



Western blot analysis of MICA/B expression in 293T (1), Jurkat (2), HeLa (3) and DLD-1 (4) using K0219-3.

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 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 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.
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.
14) Develop the film as usual. The condition for exposure and development may vary.

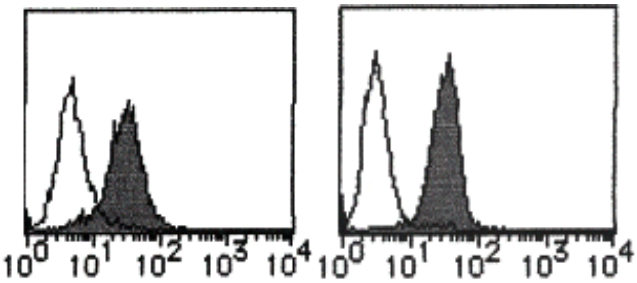
(Positive controls for Western blotting; 293T, Jurkat, HeLa and DLD-1)

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 (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 and Jurkat)



Flow cytometric analysis of MICA/B expression on 293T cells (left) and Jurkat cells (right). *Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of K0219-3 to the cells.*

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