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

MONOCLONAL ANTIBODY

# Anti-MICA/B (Human) mAb

| Code No. | Clone | Subclass | Quantity | Concentration |
|---|---|---|---|---|
| K0218-3 | BAMO3 | Mouse IgG2a 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 BAMO3) 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 Immunoprecipitation, Flow cytometry and ELISA.

**APPLICATIONS:**

Western blotting; Not recommended
Immunoprecipitation; 2-5 µg/300 µL of cell extract
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10 µg/mL (final concentration)
ELISA; 1 µg/mL (for detector antibody)

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

**SPECIES CROSS REACTIVITY:**

| Species | Human | Mouse | Rat |
|---|---|---|---|
| Cells | 293T, HeLa, Jurkat | Not tested | Not tested |
| Reactivity on IP/FCM | + | | |

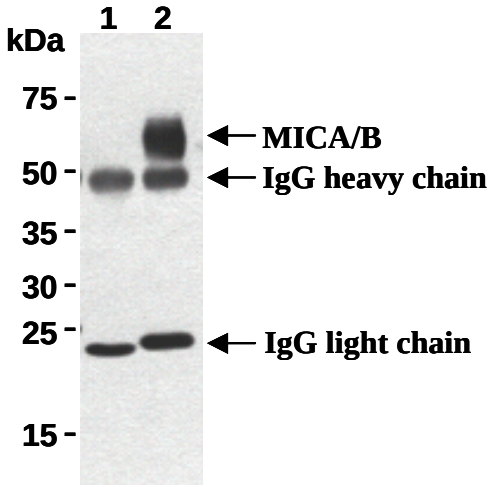
**INTENDED USE:**

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

**REFERENCES:**

1) Spreu, J., *et al., J. Immunol.* **177**, 3143-3149 (2006)
2) Boissel, N., *et al., Immunology* **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 BAMO3 is used in these references.




***Immunoprecipitation of MICA/B from HeLa cells with mouse IgG2a (1) or K0218-3 (2).*** *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with anti-MICA/B (BAMO1) (MBL; code no. K0219-3).*

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

## PROTOCOLS:

### Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% NP-40) 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.
3) Add primary antibody as suggest in the **APPLICATIONS** into 300 µL of cell extract. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the agarose in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
6) Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
7) 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.
8) 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.
9) Incubate the membrane with 1 µg/mL of Anti-MICA/B (Human) mAb (MBL; code no. K0219-3) diluted with PBS, pH 7.2 containing 1% skimmed for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
10) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
11) Incubate the membrane with 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.
12) Wash the membrane with PBS-T (5 minutes x 3 times).
13) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
14) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

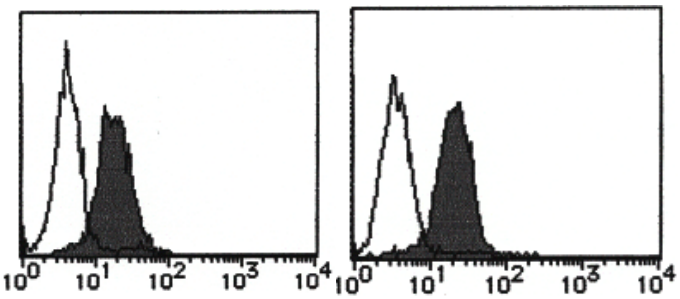
(Positive control for Immunoprecipitation; HeLa)

### 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 0.1% $NaN_3$].
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 and HeLa)




***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 K0218-3 to the cells.*

### ELISA

1) Distribute 100 µL/well of the Anti-MICA (Human) mAb or Anti-MICB (Human) mAb (1 µg/mL, MBL; code no. K0217-3, K0220-3) 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 or MICB standard (0~20 ng/mL, American Research Products, Inc.; code no. 12-4415, 12-4416) 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 (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 or 1:2,000 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.

## RELATED PRODUCTS:

K0217-3 Anti-MICA (Human) mAb (AMO1)
K0219-3 Anti-MICA/B (Human) mAb (BAMO1)
K0220-3 Anti-MICB (Human) mAb (BMO1)
M076-3 Mouse IgG2a (isotype control) (6H3)