OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for AM26693AF-N

MICA Mouse Monoclonal Antibody [Clone ID: BAMO3]

Product data:

Product Type:	Primary Antibodies
Clone Name:	BAMO3
Applications:	ELISA, FC, IP
Recommended Dilution:	**Immunoprecipitation:** 2-5 µg/300 µl of cell extract. **Flow Cytometry:** 10 µg/ml (final concentration). **ELISA:** 1 µg/ml (for detector antibody). **Western blot:** Not recommended. For more details See *Protocols below.*
Reactivity:	Human
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	MICA*01, MICA*04 and MICB*02 transfected P815 cells
Specificity:	This antibody reacts with MICA/B.
Formulation:	PBS containing 50% Glycerol, pH 7.2 State: Azide Free State: Liquid purified IgG fraction from Hybridoma (Clone BAMO3) Supernatant Preservative: None
Concentration:	lot specific
Purification:	Protein A Agarose Chromatography
Conjugation:	Unconjugated
Storage:	Upon receipt, store (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	MHC class I polypeptide-related sequence A
Database Link:	Entrez Gene 100507436 Human Q29983

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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, ï§ï¤T cells and CD8+ï¡ï¢T 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.

Synonyms: MHC class I polypeptide-related sequence A, MIC-A, PERB11.1, MHC class I polypeptide-related sequence B, MIC-B, PERB11.2

Note: This product was originally produced by MBL International.

Protocol:

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/cm2 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 (BAMO1) monoclonal antibody 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 the 1:10,000 HRP-conjugated anti-mouse IgG 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

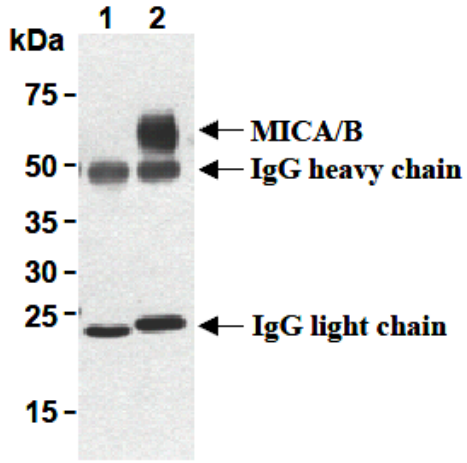
We usually use Fisher tubes or equivalents as reaction tubes for all step described below.
1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN3].
2) Resuspend the cells with washing buffer (5x10e6 cells/mL).
3) Add 50 µL of the cell suspension into each 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) 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 FITC conjugated anti-mouse IgG 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

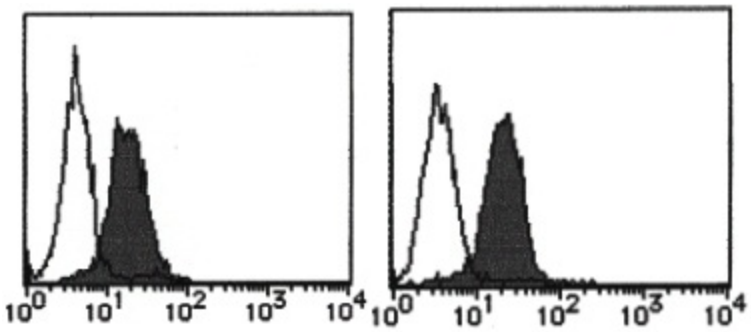
1) Distribute 100 µL/well of the anti-MICA (AMO1) or anti-MICB (BMO1) monoclonal antibody (1 µg/mL) diluted with PBS to each well.2) Incubate it overnight at 4°C.
3) Add 100 µL/well of 15% BSA/PBS.
4) Incubate it for 1 hour at 37oC.
5) Wash the plates 4 times with PBS-T [0.05% Tween-20 in PBS].
6) Distribute 100 µL/well of the samples or the recombinant MICA or MICB standard (0~20 ng/mL) 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 the anti-MICA/B monoclonal antibody (BAMO3) (1 µg/mL) 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 HRP-conjugated anti-mouse IgG2a 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.
16) Incubate it for 5~60 minutes. The condition for reaction may vary.
17) Distribute 100 µL/well of 1 M H2SO4 to each well and stop enzyme reaction.
18) After gentle mixing, determine the absorbance at 450 nm of each well by a spectrophotometer.

Product images:



Immunoprecipitation of MICA/B from HeLa cells with mouse IgG2a (1) or AM26693AF-N (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with anti MICA/B (BAMO1).



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 AM26693AF-N to the cells.