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Product datasheet for AM26571AF-N

MICA Mouse Monoclonal Antibody [Clone ID: AMO1]

Product data:

Product Type:	Primary Antibodies
Clone Name:	AMO1
Applications:	ELISA, FC
Recommended Dilution:	**Flow Cytometry:** 10 µg/ml (final concentration). **ELISA:** 1 µg/ml (capture antibody). Not recomended for Western Blot or Immunoprecipitation.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	MICA*01, MICA*04 and MICB*02 transfected P815 cells
Specificity:	This antibody reacts with MICA. The epitope was mapped to the helical surfaces of the MIC α1 α2 platform domain.
Formulation:	PBS containing 50% Glycerol, pH 7.2 State: Azide Free State: Liquid purified Ig fraction Preservative: None
Concentration:	lot specific
Purification:	Protein A Agarose Chromatography
Conjugation:	Unconjugated
Storage:	Upon receipt, store undiluted (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 ex pressed on NK (Natural Killer) cells, NKT cells, γδ T cells and CD8 + αβ 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

Note: Protocol:

Flow Cytometric analysis for floating cells

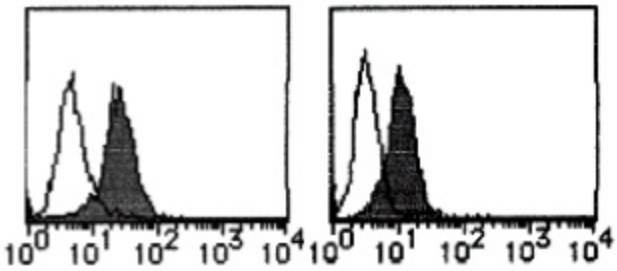
We usually use Fisher tubes or equivalents as reaction tubes for all step described below.
1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN3].
2) Resuspend the cells with washing buffer (5x10e6 cells/mL).
3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
4) Add 20 µ L 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 monoclonal antibody (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)

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 µ L/well of the 1:5,000 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 MH2SO4 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:



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 AM26571AF-N to the cells.