Product Data Sheet

CD99 / MIC2 Antibody (HO36-1 1)

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Tested Species Reactivity	Published Species Reactivity
Human (Hu)	Human (Hu)
Rat (Rt)	
Tested Applications	Dilution *
Western Blot (WB)	1:100-1:500
Immunofluorescence (IF)	1:10-1:100
Immunocytochemistry (ICC)	1:10-1:100
Immunohistochemistry (Paraffin) (IHC (P))	1:100-200
Flow Cytometry (FACS)	2 ug/test
Published Applications	Dilution
Immunohistochemistry (IHC)	See publications
* Suggested working dilutions are given as a guide only. It is rec- experiment using appropriate negative and positive controls.	ommended that the user titrates the product for use in their own

Details	
Catalog Number:	MA5-12954
Size:	500 µL
Class:	Monoclonal
Туре:	Antibody
Clone:	HO36-1.1
Host / Isotype:	Mouse / IgM
Immunogen:	Purified E-rosette forming cells from human peripheral blood lymphocytes

Form Information	
Form:	Liquid
Concentration:	0.2mg/ml
Purification:	Ammonium sulfate precipitation
Storage Buffer:	PBS, pH 7.4, with 0.2% BSA
Preservative:	0.09% sodium azide
Storage Conditions:	4° C

Product Specific Information

MA5-12954 targets CD99 in WB, IF, FACS and IHC (P) applications and shows reactivity with Human and Rat samples. This antibody is not suitable for Mouse spleen cells in Western blot analysis.

The MA5-12954 immunogen is purified E-rosette forming cells from human peripheral blood lymphocytes.

General Information

CD99, or MIC2 gene product, or E2 antigen is expressed on the cell membrane of some lymphocytes, cortical thymocytes, and granulosa cells of the ovary. The antigen is also expressed by most pancreatic islet cells, Sertoli cells of the testis, and some endothelial cells. Mature granulocytes express very little or no CD99. MIC2 is strongly expressed on Ewing's sarcoma cells and primitive peripheral neuroectodermal tumors.

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Western Blot with anti-CD99 / MIC2 Monoclonal Antibody [HO36-1.1] (MA5-12954)

Western blot analysis of CD99 was performed by loading 25 ug of Jurkat (Lane 1), THP-1 (Lane 2), and Mouse spleen (Lane 3) cell lysates and a molecular weight protein ladder onto an SDS polyacrylamide gel. Proteins were transferred to a PVDF membrane and blocked with a blocking buffer at 4°C overnight. The membrane was probed with a CD99 monoclonal antibody (Product # MA5-12954) at a dilution of 1:200 (Jurkat and Mouse spleen) and 1:100 (THP-1) overnight at 4°C, washed in TBST, and probed with an HRP-conjugated secondary antibody for 1 hr at room temperature in the dark. Results show a band at 32 kDa in Jurkat and THP-1 cell lysates.

Immunofluorescence with anti-CD99 / MIC2 Monoclonal Antibody [HO36-1.1] (MA5-12954)

Immunofluorescent analysis of CD99 (green) showing staining in the membrane of Jurkat cells (right) compared to a negative control without primary antibody (left). Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with a CD99 monoclonal antibody (Product # MA5-12954) in 3% BSA-PBS at a dilution of 1:20 and incubated overnight at 4°C in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody in PBS at room temperature in the dark. F-actin (red) was stained with a fluorescent red phalloidin and nuclei (blue) were stained with Hoechst or DAPI. Images were taken at a magnification of 60x.



Immunofluorescence with anti-CD99 / MIC2 Monoclonal Antibody [HO36-1.1] (MA5-12954)

Immunofluorescent analysis of CD99 (green) showing staining in the membrane of THP-1 cells (right) compared to a negative control without primary antibody (left). Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with a CD99 monoclonal antibody (Product # MA5-12954) in 3% BSA-PBS at a dilution of 1:20 and incubated overnight at 4°C in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody in PBS at room temperature in the dark. F-actin (red) was stained with a fluorescent red phalloidin and nuclei (blue) were stained with Hoechst or DAPI. Images were taken at a magnification of 60x.



Immunohistochemistry with anti-CD99 / MIC2 Monoclonal Antibody [HO36-1.1] (MA5-12954)

Formalin-fixed, paraffin-embedded human Ewing's sarcoma stained with CD99 antibody using peroxidase-conjugate and DAB chromogen. Note cell membrane staining of tumor cells.



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Flow cytometry analysis of CD99 in PBMC cells (green) compared to an isotype control (blue). Human blood was collected, combined with a hydrophilic polysaccharide, centrifuged, transferred to a conical tube and washed with PBS. 50 ul of cell solution was added to each tube at a dilution of 2x10^7 cells/ml, followed by the addition of 50 ul of isotype control and primary antibody (Product # MA5-12954) at a dilution of 2 ug/test. Cells were incubated for 30 min at 4°C and washed with a cell buffer, followed by incubation with a DyLight 488-conjugated secondary antibody for 30 min at 4°C in the dark. FACS analysis was performed using 400 ul of cell buffer.





Flow cytometry analysis of CD99 in Jurkat cells (green) compared to an isotype control (blue). Cells were harvested, adjusted to a concentration of 1-5x10^6 cells/ml, fixed with 2% paraformaldehyde and washed with PBS. Cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with a CD99 monoclonal antibody (Product # MA5-12954) at a dilution of 2 ug/test for 60 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a Dylight 488-conjugated secondary antibody and re-suspended in PBS for FACS analysis.

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PubMed References for CD99 / MIC2 Antibody (HO36-1.1)

3 Immunohistochemistry	References
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Immunohistochemistry (IHC)	See publications
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Formalin-fixed, paraffin-embedded human Ewing's sarcoma stained with CD99 antibody using peroxidase-conjugate and DAB chromogen. Note cell membrane staining of tumor cells.



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