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

MONOCLONAL ANTIBODY

Anti-Mouse Fas (CD95)

Code No.	Clone	Subclass	Quantity	Concentration
D027-3	RMF6	Rat IgG2a	100 µg	1 mg/mL

BACKGROUND: It is now widely accepted that apoptosis plays an important role in the selection of immature thymocytes and Ag-primed peripheral T cells. Fas antigen is a cell-surface protein that mediates apoptosis. It is expressed in various tissues including the thymus and has structural homology with a number of cell-surface receptors, including tumor necrosis factor receptor and nerve growth factor receptor.

SOURCE: This antibody was purified from ascites fluid by ammonium sulfate precipitation and affinity chromatography on protein G agarose. This hybridoma (clone RMF6) was established by fusion of mouse myeloma cell NS-1 with rat spleen cells immunized with the recombinant chimera protein of mouse soluble Fas and AIC2A.

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 mouse Fas (CD95 antigen) on Flow cytometry.

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10 µg/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Not Tested	transfectant	Not Tested
Reactivity on FCM		+	

INTENDED USE:

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REFERENCES:

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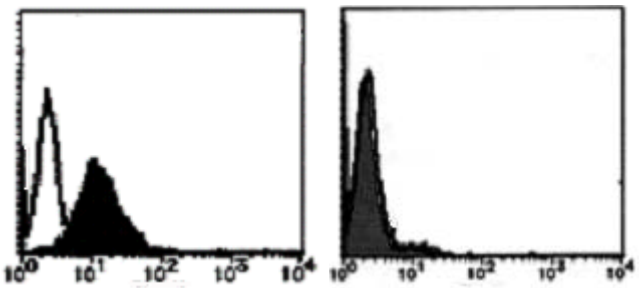
2) Xiao, S., *et al., J. Biol. Chem.* **277**, 50907-50913 (2002)
3) Yoshikawa, H., *et al., J. Immunol.* **167**, 2487-2495 (2001)
4) Raoul, C., *et al., J. Cell. Biol.* **147**, 1049-1061 (1999)
5) Gullo, C. A., *et al., J. Immunol.* **162**, 6466-6472 (1999)
6) Park, C-S., *et al., J. Immunol.* **160**, 5790-5796 (1998)
7) Biancone, L., *et al., J. Exp. Med.* **186**, 147-152 (1997)
8) Mori, T., *et al., Blood* **89**, 3565-3573 (1997)
9) Nishimura, Y., *et al., J. Immunol.* **154**, 4395-4403 (1995)

Clone RMF6 is used in these references.

RELATED PRODUCTS:

SY-001	Anti-Human Fas (CD95) (CH-11)
MD-10-3	Anti-Human Fas (CD95) (UB2)
MD-10-4	FITC labeled Anti-Human Fas (CD95) (UB2)
MD-10-5	PE labeled Anti-Human Fas (CD95) (UB2)
MD-11-3	Anti-Human Fas (CD95) (ZB4)
D026-3	Anti-Mouse Fas (CD95) (RMF2)
D041-3	Anti-Human Fas Ligand (4H9)
D041-4	FITC labeled Anti-Human Fas Ligand (4H9)
D041-5	PE labeled Anti-Human Fas Ligand (4H9)
D041-6	Biotin labeled Anti-Human Fas Ligand (4H9)
D042-3	Anti-Human Fas Ligand (4A5)
D057-3	Anti-Mouse Fas Ligand (FLIM58)
D057-4	FITC labeled Anti-Mouse Fas Ligand (FLIM58)
D057-6	Biotin labeled Anti-Mouse Fas Ligand (FLIM58)
D069-3	Anti-Mouse Fas Ligand (FLIM4)



Flow cytometric analysis of CD95 antigen expression on transfectant cells (left) and parental cells (right). *Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D027-3 to the cells.*

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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% 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^oC). Remove supernatant by careful aspiration.
4) Add 10 μL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN_3 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 with 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-rat IgG (MBL; code no. IM-0827) 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 control for Flow cytometry; transfectant)