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

MONOCLONAL ANTIBODY

Anti-Fas (CD95) (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D026-3	RMF2	Rat IgG1	100 µL	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 (clone RMF2) by ammonium sulfate precipitation and affinity chromatography on protein A. This hybridoma was established by fusion of mouse myeloma cell NS-1 with Lewis rat splenocyte immunized with recombinant chimera protein of soluble mouse 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 recognizes the Balb/c and MRL mouse Fas antigen on Flow cytometry and induces apoptosis to Fas antigen expressing murine cells.

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10 µg/mL (final concentration)
Function; Induction of apoptosis. This antibody has killing activity.

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

INTENDED USE:

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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Not tested	Transfectant	Not tested
Apoptosis		+	

REFERENCES:

1) Naka, M., *et al., Med. Mol. Morphol.* **42**, 143-149 (2009) [Function]
2) Kusakabe, K., *et al., J. Rprod. Dev.* **51**, 333-340 (2005) [Function]
3) Xiao, S., *et al., J. Biol. Chem.* **277**, 50907-50913 (2002) [Function]
4) Sawada, T., *et al., Immunology* **103**, 81-89 (2001) [Function]
5) Komano, H., *et al., Int. Immunol.* **11**, 1035-1042 (1999)
6) Suzuki, S., *et al., Clin. Exp. Immunol.* **107**, 103-111 (1997) [Function]
7) Kataoka, T., *et al., J. Immunol.* **156**, 3678-3686 (1996)
8) Nishimura, Y., *et al., J. Immunol.* **154**, 4395-4403 (1995)

Clone RMF2 is used in these references.

PROTOCOLS:

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 0.09% NaN_3].
   *Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
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 10 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.09% 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 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 FITC conjugated anti-rat IgG antibody 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)

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Induction of apoptosis

1) $2x10^4$ cells/50 μL of L5178Y cells or mouse Fas transfected L5178Y cells was cultured in 96-well microplate at 37°C in 5% CO_2 incubator with RPMI 1640 containing 10% fetal calf serum.
2) Add 50 μL of 7.8-4,000 ng/mL Anti-Fas (CD95) (Mouse) mAb (D026-3) diluted with RPMI 1640 containing 10% fetal calf serum.
3) Cultured for appropriate times at 37°C in 5% CO_2 incubator with RPMI 1640 containing 10% fetal calf serum.
4) Cell viability was calculated by XTT assay.

RELATED PRODUCTS:

SY-001	Anti-Fas (CD95) mAb (CH-11)
MD-10-3	Anti-Fas (CD95) (Human) mAb (UB2)
MD-10-4	Anti-Fas (CD95) (Human) mAb-FITC (UB2)
MD-10-5	Anti-Fas (CD95) (Human) mAb-PE (UB2)
MD-10-A48	Anti-Fas (CD95) (Human) mAb -Alexa Fluor® 488 (UB2)
MD-11-3	Anti-Fas (CD95) (Human) mAb (ZB4)
D027-3	Anti-Fas (CD95) (Mouse) mAb (RMF6)
D041-3	Anti-Fas Ligand (CD178) (Human) mAb (4H9)
D041-4	Anti-Fas Ligand (CD178) (Human) mAb -FITC (4H9)
D041-5	Anti-Fas Ligand (CD178) (Human) mAb-PE (4H9)
D041-6	Anti-Fas Ligand (CD178) (Human) mAb -Biotin (4H9)
D042-3	Anti-Fas Ligand (CD178) (Human) mAb (4A5)
D057-3	Anti-Fas Ligand (CD178) (Mouse) mAb (FLIM58)
D057-4	Anti-Fas Ligand (CD178) (Mouse) mAb-FITC (FLIM58)
D057-6	Anti-Fas Ligand (CD178) (Mouse) mAb-Biotin (FLIM58)
D069-3	Anti-Fas Ligand (CD178) (Mouse) mAb (FLIM4)
5255	sFas Ligand ELISA Kit
M075-3	Mouse IgG1 (isotype control)
MTG-001	Clear Back