

MONOCLONAL ANTIBODY

Anti-Fas (CD95) (Human) mAb

| Code No. | Clone | Subclass | Quantity | Concentration |
|----------|-------|------------|----------|---------------|
| MD-10-3 | UB2 | Mouse 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 UB2) by ammonium sulfate precipitation and affinity chromatography on protein A agarose. This hybridoma was established by fusion of mouse myeloma cell NS-1 with Balb/c mouse splenocyte immunized with recombinant human Fas.

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 human Fas antigen specifically. Clone UB2 does not recognize the mouse Fas antigen.

APPLICATIONS:

Western blotting: Not tested

Immunoprecipitation: Not tested

Immunohistochemistry: 10-20 µg/mL

It is reported that this monoclonal antibody can be used in frozen sections and paraffin embedded sections in reference number 1), 11) and 12), respectively.

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 |
|-------------------|--------------|--------------|------------|
| Cells | Transfectant | Transfectant | Not tested |
| Reactivity on FCM | + | - | |

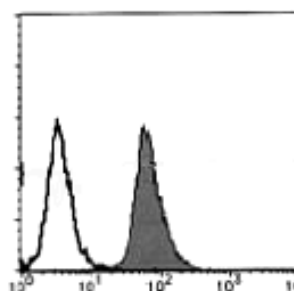
INTENDED USE:

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

REFERENCES:

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- 13) Dai, C. H., *et al.*, *Blood* **91**, 1235-1242 (1998)
- 14) Ando, K., *et al.*, *J. Immunol.* **158**, 5283-5291 (1997)
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- 18) Ito, N., *et al.*, *Cell.* **66**, 233-243 (1991)
- 19) Kobayashi, N., *et al.*, *PNAS.* **87**, 9620-9624 (1990)
- 20) Yonehara, S., *et al.*, *J. Exp. Med.* **169**, 1747-1756 (1989)

Clone UB2 is used in reference number 1) - 16).



Flow cytometric analysis of human Fas expression on transfectant

- MD-10-3
- isotype control

PROTOCOL:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

- 1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN₃].

*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 (5×10^6 cells/mL).
- 3) Add 50 μ L 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, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
- 5) Add 40 μ L of the primary antibody at the concentration 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 μ L of 1:100 Anti-IgG (Mouse) pAb-FITC (MBL; code no. 238) 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 μ L of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Transfectant)

RELATED PRODUCTS:

| | |
|-----------|---|
| SY-001 | Anti-Fas (CD95) mAb (CH-11) |
| 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) |
| D026-3 | Anti-Fas (CD95) (Mouse) mAb (RMF2) |
| 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 |