

MONOCLONAL ANTIBODY

# Alexa Fluor<sup>®</sup> 488 labeled Anti-Fas/CD95

Code No.	Clone	Subclass	Quantity	Concentration
MD-10-A48	UB2	Mouse IgG1	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 (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 1% BSA and 0.09% NaN<sub>3</sub>.

\*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.

**STORAGE:** This antibody is stable for one year from the date of shipment when stored at 4°C.

**REACTIVITY:** This antibody recognizes the human Fas antigen specifically. Clone UB2 does not recognize the mouse Fas antigen.

**APPLICATION:**

Flow cytometry: 10 µg/mL (final concentration)

\*Please refer to the data sheet (MBL code no. MD-10-3) for other applications.

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

**SPECIES CROSS REACTIVITY:**

Species	Human	Mouse	Rat
Cells	lymphocyte, monocyte, granulocyte, transfectant	transfectant	Not Tested
Reactivity on FCM	+	-	

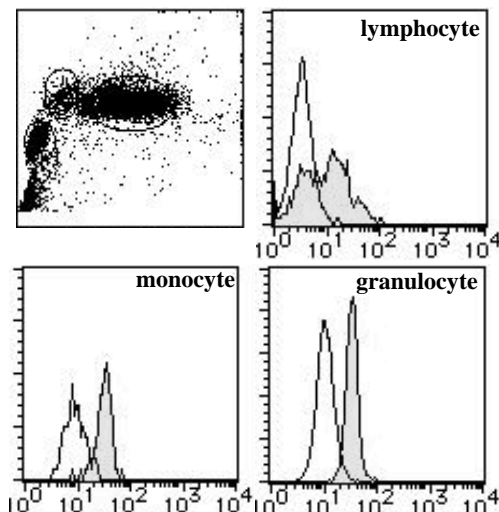
**INTENDED USE:**

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

**REFERENCES:**

- 1) Boula, A., *et al.*, *Clin. Cancer Res.* **12**, 3099-3108 (2006)
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- 10) Kuwano, K., *et al.*, *Am. J. Respir. Cell Mol. Biol.* **20**, 53-60 (1999)
- 11) Dai, C. H., *et al.*, *Blood* **91**, 1235-1242 (1998)
- 12) Ando, K., *et al.*, *J. Immunol.* **158**, 5283-5291 (1997)
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- 14) Hata, H., *et al.*, *Blood* **86**, 1939-1945 (1995)
- 15) Watanabe-Fukunaga, R., *Nature* **356**, 314-317 (1992)
- 16) Ito, N., *et al.*, *Cell.* **66**, 233-243 (1991)
- 17) Kobayashi, N., *et al.*, *PNAS.* **87**, 9620-9624 (1990)
- 18) Yonehara, S., *et al.*, *J. Exp.Med.* **169**, 1747-1756 (1989)

Clone UB2 is used in reference number 1) - 14).



**Flow cytometric analysis of Fas expression on peripheral blood lymphocyte, monocyte and granulocyte.** Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of MD-10-A48 to the cells.

## PROTOCOLS:

### Flow cytometric analysis for whole blood cells

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

- 1) Add 50  $\mu$ L of the primary antibody at the concentration as suggested in the **APPLICATION** diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1%  $\text{NaN}_3$ ] into each tube.
- 2) Add 50  $\mu$ L of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25 °C).
- 3) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 4) Lyse with OptiLyse C (for analysis on Beckman Coulter instruments) or OptiLyse B (for analysis on BD instruments), using the procedure recommended in the respective package inserts.
- 5) Add 1 mL of  $\text{H}_2\text{O}$  to each tube and incubate for 10 minutes at room temperature.
- 6) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 7) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 8) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; lymphocyte, monocyte, granulocyte)

### 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 0.1%  $\text{NaN}_3$ ].
- 2) Resuspend the cells with washing buffer (5 x 10<sup>6</sup> cells/mL).
- 3) Add 50  $\mu$ 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  $\mu$ 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 20  $\mu$ L of the primary antibody at the concentration as suggested in the **APPLICATION** 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) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer.

## RELATED PRODUCTS:

D038-3	anti-Bcl-2 (83-8B)
D038-5	PE labeled anti-Bcl-2 (83-8B)
K0154-3	anti-mouse Bcl-2 (10C4)
M010-3	anti-Bax (4F11)
K0151-3	anti-Bax (5B7)
K0152-3	anti-Bax (6A7)
K0153-3	anti-Bcl-xL (2H12)
M073-3	anti-Caspase-2 (4F8)
M097-3	anti-Caspase-3 (1F3)
K0197-3	anti-Caspase-3 (AMI-3-1-11)
M087-3	anti-Caspase-3 (1F9)
M088-3	anti-Caspase-3 (7D12)
M029-3	anti-Caspase-4 (4B9)
M060-3	anti-Caspase-5 (4F7)
M070-3	anti-Caspase-6 (3E8)
M053-3	anti-Caspase-7 (4G2)
M032-3	anti-Caspase-8 (5F7)
M058-3	anti-Caspase-8 (5D3)
M054-3	anti-Caspase-9 (5B4)
M059-3	anti-Caspase-10 (4C1)
K0206-3	anti-Caspase-12 (14F7)
K0207-3	anti-Caspase-12 (14F4)
K0193-3	anti-Caspase-14 (1-71)
M028-3	anti-Mouse TRAF1 (3D4)
M030-3	anti-Bag-1 (4A2)
M031-3	anti-TRADD (3E11)
M033-3	anti-FADD (1F7)
M035-3	anti-FADD (4G3)
M037-3	anti-DFF45/ICAD (6B8)
M044-3	anti-XIAP (2F1)
M056-3	anti-RAIDD (4B12)
M072-3	anti-BID (5C9)
M074-3	anti-Apaf-1 (5C1)
M083-3	anti-AcinusL (3H8)
M112-3	anti-mouse TRAF2 (6F8)
592	anti-mouse TRAF2 (polyclonal)
597	anti-mouse TRAF6 (polyclonal)
M092-3	anti-TRAF6 (1F8)
SY-001	anti-Fas/CD95 (CH-11)
MD-10-3	anti-Fas/CD95 (UB2)
D026-3	anti-mouse Fas/CD95 (RMF2)
D027-3	anti-mouse Fas/CD95 (RMF6)
D041-3	anti-human FasL/CD178 (4H9)
D041-4	FITC labeled anti-human FasL/CD178 (4H9)
D041-5	PE labeled anti-human FasL/CD178 (4H9)
D041-6	Biotin labeled anti-human FasL/CD178 (4H9)
D042-3	anti-human FasL/CD178 (4A5)
D057-3	anti-mouse FasL/CD178 (FLIM58)
D057-4	FITC labeled anti-mouse FasL/CD178 (FLIM58)
D057-6	Biotin labeled anti-mouse FasL/CD178 (FLIM58)
D069-3	anti-mouse FasL/CD178 (FLIM4)
D086-3	anti-ASC (23-4)
D132-3	CD279/PD-1 (J110)
D132-4	FITC labeled CD279/PD-1 (J110)
D133-3	CD279/PD-1 (J105)
D230-3	CD274/PD-L1 (27A2)
D231-3	Mouse CD273/PD-L2 (54-1)

D161-3 anti-MFG-E8 (2422)  
D199-3 anti-MFG-E8 (18A2-G10)  
D184-3 anti-Granulysin (RB1)  
D185-3 anti-Granulysin (RC8)  
D185-6 Biotin labeled anti-Granulysin (RC8)  
D186-3 anti-Granulysin (RF10)  
D200-3 CD257/BAFF/BLyS (1D6)  
D200-4 FITC labeled CD257/BAFF/BLyS (1D6)  
D201-3 CD268/BAFF-R/BR3 (8A7)  
D201-4 FITC labeled CD268/BAFF-R/BR3 (8A7)  
D201-5 PE labeled CD268/BAFF-R/BR3 (8A7)  
K0033-3 anti-DR3 (B65)  
K0033-4 FITC labeled anti-DR3 (B65)  
K0039-3 CD120a/TNFR1 (H398)  
K0039-4 FITC labeled CD120a/TNFR1 (H398)  
K0040-3 CD120b/TNFR2 (80M2)  
K0040-4 FITC labeled CD120b/TNFR2 (80M2)  
K0040-5 PE labeled CD20b/TNFR2 (80M2)  
K0127-3 anti-Daxx (DAXX-01)  
K0145-3 anti-CD30 (Ber-H2)  
K0145-4 FITC labeled anti-CD30 (Ber-H2)  
K0157-3 anti-IKK $\gamma$  (I- $\kappa$ B Kinase  $\gamma$ ) (DA10-12)  
K0159-3 anti-IKK $\gamma$  (I- $\kappa$ B Kinase  $\gamma$ ) (EA2-6)  
K0194-3 anti-HtrA2/Omi (18-1-83)  
CM001-1 anti-Cytochrome c (1E4)  
PM004 anti-Smac/DIABLO (polyclonal)  
PD005 anti-Vimentin Fragment (V1) (polyclonal)  
PD006 anti-SET $\beta$  (p41/p42) (polyclonal)  
PD007 anti-SET $\beta$  (p42) (polyclonal)  
PD008 anti-SET $\beta$  (p41) (polyclonal)  
591 anti-Bad (polyclonal)  
M075-A48 Alexa Fluor<sup>®</sup> 488 labeled Mouse IgG1 isotype control (2E12)

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