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

MBL
A JSR Life Sciences Company

MONOCLONAL ANTIBODY

Anti-Fas (CD95) (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
SY-001	CH-11	Mouse IgM	100 µL	500 µg/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 using affinity chromatography. This hybridoma (clone CH-11) was established by fusion of mouse myeloma cell NS-1 with Balb/c mouse splenocyte immunized with the human diploid fibroblast FS-7 cell line.

FORMULATION: 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.

SPECIFICITY: This antibody recognizes the human Fas antigen and does not recognize the mouse Fas antigen.

REACTIVITY: This antibody induces apoptosis in human cell lines expressing Fas antigen.

APPLICATIONS:

Western blotting; 20 µg/mL
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 20 µg/mL (final concentration)
Function; Induction of apoptosis. This antibody at the concentration of 100 ng/mL induces the apoptosis after 2 hours incubations at 37°C and almost all of the cells are killed after 15 hours.

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

INTENDED USE:

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

REFERENCES:

1) Yamaji, T., *et al.*, *J. Biol. Chem.* **285**, 35505-35518 (2010)
2) Arokium, H., *et al.*, *J. Virol.* **83**, 11283-11297 (2009)
3) Pallasch, C. P., *et al.*, *Blood* **112**, 4213-4219 (2008)
4) Conus, S., *et al.*, *J. Exp. Med.* **205**, 685-698 (2008)
5) Okamoto, K., *et al.*, *Genes Cells* **11**, 177-191 (2006)
6) Ohgushi, M., *et al.*, *Mol. Cell. Biol.* **25**, 10017-10028 (2005)
7) Kotone-Miyahara, Y., *et al.*, *J. Leukoc. Biol.* **76**, 1047-1056 (2004)
8) Lee, K-K., *et al.*, *J. Biol. Chem.* **276**, 19276-19285 (2001)
9) Ichikawa, K., *et al.*, *Int. Immunol.* **12**, 555-562 (2000)
10) Tsukumo, S. I., *et al.*, *Genes Cells* **4**, 541-549 (1999)
11) Yamashita, K., *et al.*, *Blood* **93**, 674-685 (1999)
12) Ito, N., *et al.*, *Cell* **66**, 233-243 (1991)
13) Kobayashi, N., *et al.*, *PNAS* **87**, 9620-9624 (1990)
14) Yonehara, S., *et al.*, *J. Exp. Med.* **169**, 1747-1756 (1989)

As this antibody is really famous all over the world, a lot of researches have been reported. These references are a part of such reports.

RELATED PRODUCTS:

Please visit our website at https://ruo.mbl.co.jp/.

The descriptions of the following protocols are examples.

Each user should determine the appropriate condition.

PROTOCOLS:

SDS-PAGE & Western blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacturer's manual for

precise transfer procedure.

6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
9) Incubate the membrane with the 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (5 minutes x 6 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
12) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Jurkat, transfectant)

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 (5 x 10^6 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 Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) 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 as suggested 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-mouse 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 controls for Flow cytometry; Jurkat, transfectant)

Apoptosis induction

1) $2x10^4$ cells/50 µL of Jurkat cells or WR19L12a cells (human Fas transfectant) 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 200 ng/mL Anti-Fas (CD95) (Human) mAb (SY-001) 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 WST-1 assay.