

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:

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- 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)
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- 11) Yamashita, K., *et al.*, *Blood* **93**, 674-685 (1999)
- 12) Ito, N., *et al.*, *Cell* **66**, 233-243 (1991)
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- 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.

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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/cm² 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% 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 x 10⁶ 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% NaN₃ 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) 2x10⁴ cells/50 µL of Jurkat cells or WR19L12a cells (human Fas transfectant) was cultured in 96-well microplate at 37°C in 5% CO₂ 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% CO₂ incubator with RPMI 1640 containing 10% fetal calf serum.
- 4) Cell viability was calculated by WST-1 assay.