

MONOCLONAL ANTIBODY

Anti-Fas (CD95) (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
MD-11-3	ZB4	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 (also known as CD95/APO-1) is a cell surface protein belonging to the tumor necrosis factor receptor superfamily, which is expressed in a variety of normal and neoplastic cells. Binding of FasL to Fas or crosslinking of Fas by anti-Fas monoclonal antibodies results in rapid induction of apoptosis in Fas expressing cells. Clone ZB4 is known as Fas blocking, Fas neutralizing or Fas antagonistic antibody, whereas anti-Fas antibody (clone CH-11) is known as the agonistic antibody.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma (clone ZB4) 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 reacts with human Fas on Western blotting and Flow cytometry. It does not react with mouse Fas.

APPLICATIONS:

Western blotting: 5 µg/mL

Immunoprecipitation: Not tested

Immunohistochemistry: Not tested

Immunocytochemistry: Not tested

Flow cytometry: 5-20 µg/mL (final concentration)

Neutralizing activity: This clone ZB4 inhibits Anti-Fas (CD95) mAb (clone CH-11, MBL, code no. SY-001) -induced apoptosis when the cells are pretreated with ZB4 for 1 hour at the concentration of 10-500 ng/mL in medium.

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

INTENDED USE:

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

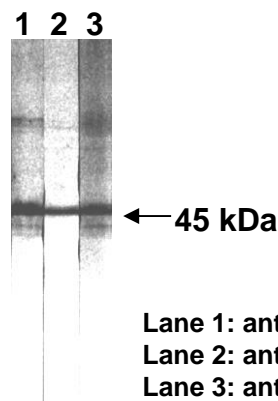
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Lymphocyte, transfectant	Not tested	Not tested
Reactivity on FCM	+		

REFERENCES:

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- 11) Jiang, S., *et al.*, *Invest. Ophthalmol. Vis. Sci.* **41**, 645-655 (2000)
- 12) Landowski, T. H., *et al.*, *Blood* **94**, 265-274 (1999)
- 13) Agrawal, S., *et al.*, *J. Clin. Invest.* **102**, 1715-1723 (1998)
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As clone ZB4 is really famous all over the world, a lot of researches have been reported. These references are a part of such reports.



Western blotting analysis of human Fas expression in transfectant

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 3 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 manufacture'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 the conditions.)
- 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
- 9) Incubate the membrane with 1:10,000 of 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 (10 minutes x 3).
- 11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
- 12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 13) Expose to an X-ray film in a dark room for 3 minutes.
- 14) Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; 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 (5x10⁶ 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) 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 washing buffer. Mix well and incubate for 30 minutes at room temperature.
- 6) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 7) Add 30 µL of 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 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; Human lymphocyte)



Flow cytometric analysis of human Fas expression on normal human lymphocyte

- Anti-Fas (CD95) (Human) mAb (MBL, code no. MD-11-3)
- isotype control

Flow cytometric analysis for whole blood cells

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

- 1) Add 50 μ L of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3] into each tube.
- 2) Add 50 μ 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) Add 30 μ L of FITC-conjugated anti-mouse IgG antibody diluted with washing buffer. Mix well and incubate for 15 minutes at room temperature.
- 5) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 6) 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.
- 7) Add 1 mL of H_2O to each tube and incubate for 10 minutes at room temperature.
- 8) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 9) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 10) Resuspend the cells with 500 μ L of the washing buffer and analyze by a flow cytometer.

Neutralizing activity

- 1) 2×10^4 cells/50 μ L of Jurkat cells were cultured in 96-well microplate.
- 2) Add 50 μ L of 500, 250, 125, 62.5, 31.3, 0 ng/mL Anti-Fas (CD95) (human) mAb (clone ZB4, MBL, code no. MD-11-3) diluted with RPMI1640 in 10% FCS to 96-well microplate.
- 3) Incubate the cells for 4 hours in CO_2 incubator at 37°C.
- 4) Add 50 μ L of 500, 125, 31.3, 0 ng/mL Anti-Fas (CD95) mAb (clone CH-11, MBL, code no. SY-001) diluted with RPMI1640 in 10% FCS to 96-well microplate.
- 5) Incubate the cells for 18 hours in CO_2 incubator at 37°C.
- 6) Cell viability was calculated by WST-1 assay.

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