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

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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10) Maedler, K., *et al.*, *Diabetes* **50**, 1683-1690 (2001)
11) Jiang, S., *et al., Invest. Ophthalmol. Vis. Sci.* **41**, 645-655 (2000)
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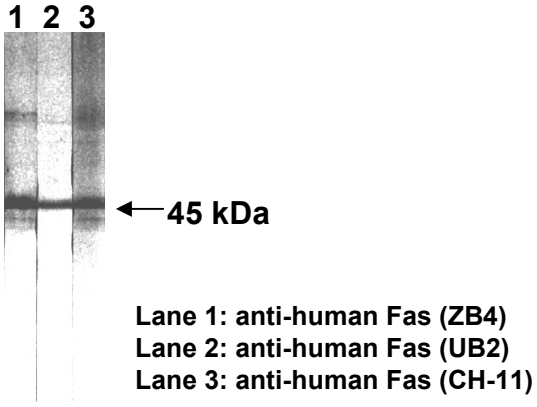
As clone ZB4 is really famous all over the world, a lot of researches have been reported. These references are a part of such reports.




***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/cm$^2$ 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.

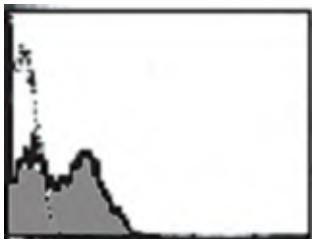
(Positive control for Western blotting; Transfectant)

### 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% $NaN_3$].
   *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 ($5x10^6$ 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) 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 µL 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 µL 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 µL 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 µL of the washing buffer and analyze by a flow cytometer.

**Neutralizing activity**

1) 2 x$10^4$ cells/50 µL of Jurkat cells were cultured in 96-well microplate.
2) Add 50 µL 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 µL 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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