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Product datasheet for AM26672AF-N

CD95 (FAS) Mouse Monoclonal Antibody [Clone ID: ZB4]

Product data:

Product Type:	Primary Antibodies
Clone Name:	ZB4
Applications:	FC, Neutralize, WB
Recommended Dilution:	**Western blot:** 5 µg/ml for chemiluminescence detection system. **Flow cytometry:** 5-20 µg/ml (final concentration). **Neutralizing activity:** This clone ZB4 inhibits the anti-Fas antibody (clone CH-11) -induced apoptosis when the cells are pretreated with ZB4 for 1 hour at a concentration of 10-500 ng/ml in medium. Detailed procedure is provided in the protocols below.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Recombinant human Fas
Specificity:	This antibody reacts with Fas.
Formulation:	PBS containing 50% glycerol, pH 7.2 State: Azide Free State: Liquid Ig fraction containing no preservatives
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Store (in aliquots) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	Fas cell surface death receptor
Database Link:	Entrez Gene 355 Human P25445

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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-human Fas antibody (clone CH-11) is known as the agonistic antibody.

Synonyms: FASLG receptor, Apo-1 antigen, APT1, FAS1, TNFRSF6

Note: This product was originally produced by MBL International.

Protocol:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volumes 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 4oC 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 4oC 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 an 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% MeOH). 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 4oC.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody will depend on the condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG 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 times). 11) Wipe excess buffer from the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with a 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 conditions 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 steps described below.

1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN3].
2) Resuspend the cells with washing buffer (5x10e6 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 (20~25oC). Remove supernatant by careful aspiration.
4) Add 10 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% 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 30 µL of 1:100 FITC conjugated anti-mouse IgG 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.

Flow cytometric analysis for whole blood cells

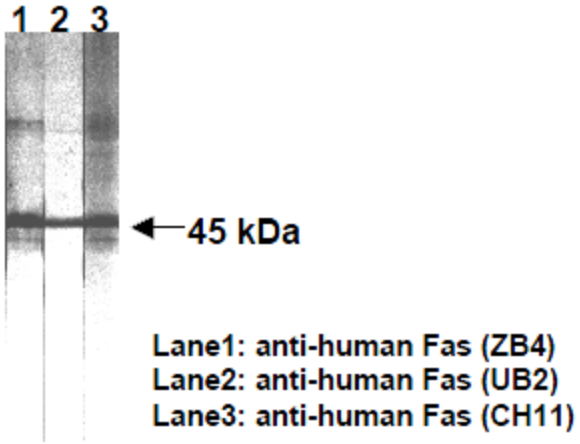
We usually use Falcon tubes or equivalents as reaction tubes for all steps described below.
1) Add 50 µL of the primary antibody at the concentration as suggested in the APPLICATIONS diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN3] into each tube.
2) Add 50 µL of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25 oC).
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 1:100 FITC conjugated anti-mouse IgG 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 H2O 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

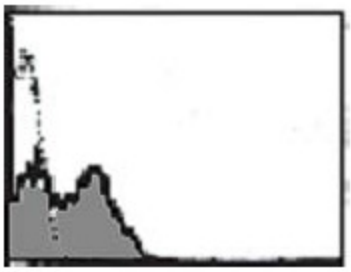
1) 20,000 cells/50 µL of WR19L12a (human Fas transfectant) cell was cultured in 96-well microplate.
2) Add 50 µL of 500, 250, 125, 62.5, 31.3, 0 ng/mL Anti-human Fas monoclonal antibody diluted with RPMI1640 in 10% FCS to 96-well microplate.
3) Incubate the cells for 4 hours in CO2 incubator at 37°C.

4) Add 50 µL of 500, 125, 31.3, 0 ng/mL Anti-human Fas monoclonal antibody diluted with RPMI1640 in 10% FCS to 96-well microplate.
5) Incubate the cells for 18 hours in CO2 incubator at 37°C.
6) Cell viability was calculated by WST-1 assay.

Product images:



Western blot analysis of human Fas expression in human Fas transfectant.



Flow cytometric analysis of human Fas expression on normal human lymphocyte. Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of [AM26672AF-S] to the cells.