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Not for use in diagnostic procedures.

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MONOCLONAL ANTIBODY

Anti-FADD mAb

Code No.	Clone	Subclass	Quantity	Concentration
M033-3	1F7	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: FADD (Fas-associated death domain protein)/MORT1 has been identified as a protein that associates specifically with the intracellular domain of Fas. It contains death domain (DD) and death effecter domain (DED) as well. DD is found in several death-inducing receptors of the TNF family, including Fas (CD95/APO-1) and TNFR-1. DED is found at N-terminus of FADD and it also present within the ICE-like protease, caspase-8 (FLICE/Mch5/MACH) and caspase-10 (FLICE2). Both DD and DED are able to associate with homologous regions in other proteins, and thus prompt binding of such proteins to one another. Upon activation Fas, it relays death signals through DD, which interacts with the DD of the adaptor molecules FADD, recruiting them to the membrane. FADD then associates with caspase-8 through DED, leading to the assembly of a death-inducing signaling complex (DISC). DISC-associated caspase-8 subsequently initiates proteolytic activation of other caspase, which in turn leads to apoptosis.

SOURCE: This antibody was purified from hybridoma (clone 1F7) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the recombinant full-length human FADD (1-208 aa).

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 and mouse FADD on Western blotting.

APPLICATIONS:
Western blotting; 1 µg/mL
Immunoprecipitation; Not tested*
*It is reported that this antibody can be used in this application in the reference number 4).
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat, Raji, HeLa, U937, ZR-75-1, A431, HL-60	HUC-Fm, WR19L, NIH/3T3	Not tested
Reactivity on WB	+	+	

INTENDED USE:
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14) Muzio, M., *et al., Cell* **85**, 817-827 (1996)
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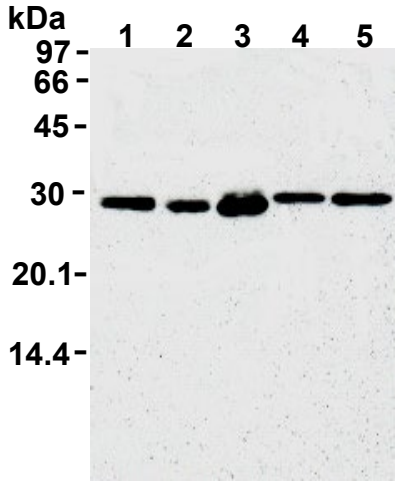
Clone 1F7 is used in the reference number 1) - 12).

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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 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 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 6).
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 (5 minutes x 6).
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, HeLa, ZR-75-1, NIH/3T3 and WR19L)



Western blotting analysis of FADD expression in Jurkat (1), HeLa (2), ZR-75-1 (3), NIH/3T3 (4) and WR19L (5) using M033-3.

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