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Product datasheet for AM26589AF-N

FADD (1-208) Mouse Monoclonal Antibody [Clone ID: 1F7]

Product data:

Product Type:	Primary Antibodies
Clone Name:	1F7
Applications:	WB
Recommended Dilution:	**Western blot:** 1 µg/ml for chmiluminescence detection system. For details see protocol below.
Reactivity:	Human, Mouse
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Recombinant full-length human FADD (1-208 aa)
Specificity:	This antibody reacts with FADD.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	Fas associated via death domain
Database Link:	Entrez Gene 8772 Human Q13158

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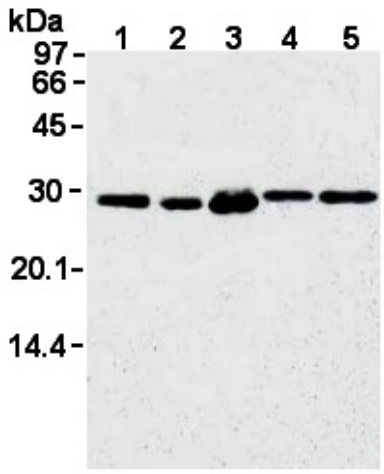
Background: FADD (Fas-associated death domain protein)/MORT1 has been identified as a protein that associates specifically with the intracellular domain of Fas. It contains death domain (D D) and death effecter domain (DED) as well. DD is found in several death-inducing receptors of the TNF family, including Fas (CD95/APO-1) and TNFR-1. DED is found at N-terminus of FADD and it also present within the IC E-like protease, caspase-8 (FLICE/Mch5/MACH) and caspase-10 (FLICE2). Both DD and DED are able to a ssociate 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, whic h interacts with the DD of the adaptor molecules FADD, recruiting them to the membrane. FADD then associat es 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.

Synonyms: FAS-associated death domain protein, MORT1, GIG3

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 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 o 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 o 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 µ L 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% 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 4 o C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest 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 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 (5 minutes x 6 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemilumin escence 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, WR19L)

Product images:



Western blot analysis of FADD expression in Jurkat (1), HeLa (2), ZR-75-1 (3), NIH/3T3 (4) and WR19L (5) using AM26589AF-N.