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MONOCLONAL ANTIBODY

Anti-Caspase-8 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M032-3	5F7	Mouse IgG2b	100 µL	1 mg/mL

BACKGROUND: Caspase-8 (FLICE/MACH/Mch5) is a member of the ICE (interleukin-1 converting enzyme)/CED-3 family cysteine protease. It is the most upstream protease that receives the activation signal from the Fas (APO1/CD95) and TNFR1 (Tumor Necrosis Factor Receptor 1) to initiate the apoptotic protease cascade that leads to activation of ICE/CED-3 family proteases. Caspase-8 has high homologous region to the ICE/CED-3 family in C-terminal and two death effecter domains (DED) in N-terminal. Binding of caspase-8 to FADD (MORT1) through association of their DED, and consequent activation of the caspases by their proteolytic cleavage, are thought to be critical steps in the initiation of Fas- and TNFR1-induced apoptosis. Recently the inhibitor of Fas- and TNFR1-induced apoptosis is identified, called I-FLICE (FLIP/Casper/FLAME/CASH). I-FLICE has high homology to caspase-8 and it contains two DED, which interacts with caspase-8 and FADD, and potently inhibits Fas- and TNFR1-induced apoptosis.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma was established by fusion of mouse myeloma P3U1 with Balb/c mouse splenocyte immunized with the recombinant human FLICE corresponding to C-terminal amino acids (176-460 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 caspase-8 on Western blotting. It doesn't cross-react with other caspases including caspase-10 (FLICE2) which has high homology to caspase-8, however cross-reactivity with I-FLICE has not been examined.
This antibody detects 55 kDa of human caspase-8a (MACH α1) as well as 54 kDa of human caspase-8b (MACH α2) on Western blotting with total cell lysate from Jurkat, Raji, U937 and HeLa. Occasionally, unidentified 72 kDa band might be detected on Western blotting in some cell lines.
This antibody also detects 43 kDa of cleaved intermediate and 18 kDa of activated large subunit of caspase-8.

APPLICATIONS:

Western blotting; 1 µg/mL
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunofluorescence; Not tested*
*It is reported that this antibody can be used in immunofluorescence in the reference number 7).
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, U937, HeLa	NIH/3T3, WR19L, Ba/F3	Not tested
Reactivity on WB	+	-	

INTENDED USE:

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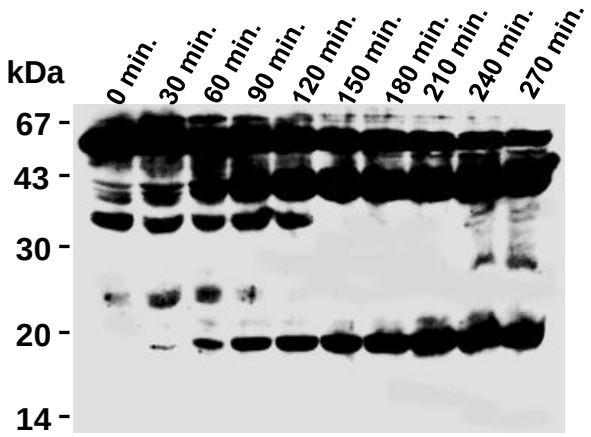
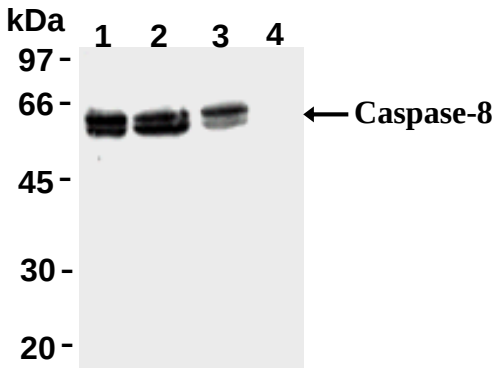
As clone 5F7 is famous all over the world, a lot of researches have been reported. These references are a part of such reports.



Western blotting analysis of Caspase-8 fragments in apoptosis induced Jurkat cells using M032-3.
The Jurkat cells were treated with anti-Fas monoclonal antibody (clone CH-11, MBL, code no. SY-001) for the indicated time.



Western blotting analysis of human Caspase-8 expression in Jurkat (1), Raji (2), HeLa (3) and WR19L (4) using M032-3.

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 2 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 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 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 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Jurkat, Raji and HeLa)

Apoptosis induction

1) 2 x 10^4 cells/50 µL of Jurkat cells are cultured in 96-well microplate at 37°C in 5% CO_2 incubator with RPMI 1640 containing 10% fetal calf serum.
2) Add 50 µL of 200 ng/mL Anti-Fas (CD95) mAb (clone CH-11, MBL, code no. SY-001) diluted with RPMI 1640 containing 10% fetal calf serum.
3) Culture for appropriate times at 37°C in 5% CO_2 incubator with RPMI 1640 containing 10% fetal calf serum.

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