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

MBL
A JSR Life Sciences Company

MONOCLONAL ANTIBODY

Anti-Caspase-8 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M058-3	5D3	Mouse IgG2b κ	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 (clone 5D3) was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the recombinant human caspases-8 corresponding to C-terminal amino acids (180-480 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 with total cell lysate from human cell line. This antibody also detects 43 kDa of cleaved intermediate and 18 kDa of activated large subunit of caspase-8.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat, Raji, U937, HeLa, MCF7, HEp-G2, ZR-75-1	WR19L	PC12
Reactivity on WB	+	-	-

APPLICATIONS:

Western blotting; 1 µg/mL
Immunoprecipitation; 1 µg/200 µL of cell extract from 2.5 x 10^6 cells

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

INTENDED USE:

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

REFERENCES:

1) Huang, C. R., *et al., Anticancer Res.* **30**, 2065-2071 (2010) [WB]
2) Phillips, D. C., *et al*., *Cancer Res*. **67**, 756-764 (2007) [WB]
3) Tsuruma, K., *et al., J. Biol. Chem*. **281**, 11397-11404 (2006) [WB]
4) Martin, S., *et al., Cancer Res*. **65**, 11447-11458 (2005) [WB]
5) O'Reilly, L. A., *et al., Cell Death Differ.* **11**, 724-736 (2004) [WB]
6) Cryns, V., *et al*., *Genes Dev.* **12**, 1551-1570 (1998)
7) Nicholson, D. W., *et al*., *Trends Biochem. Sci.* **22**, 299-306 (1997)
8) Cohen, G. M., *Biochem. J.* **326**, 1-16 (1997)
9) Muzio, M., *et al., J. Biol. Chem.* **272,** 2952-2956 (1997)
10) Hu, S., *et al., J. Biol. Chem.* **272,** 17255-17257 (1997)
11) Irmler, M., *et al., Nature* **388,** 190-195 (1997)
12) Scaffidi, C., *et al., J. Biol. Chem.* **272,** 26953-26958 (1997)
13) Duan, H., *et al*., *J. Biol. Chem.* **271**, 16720-16724 (1996)
14) Boldin, M. P., *et al., Cell* **85,** 803-815 (1996)
15) Muzio, M., *et al., Cell* **85,** 817-827 (1996)
16) Arends, M. J., *et al*., *Int. Rev. Exp. Pathol.* **32**, 223-254 (1991)
Clone 5D3 is used in reference number 1) - 5).

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

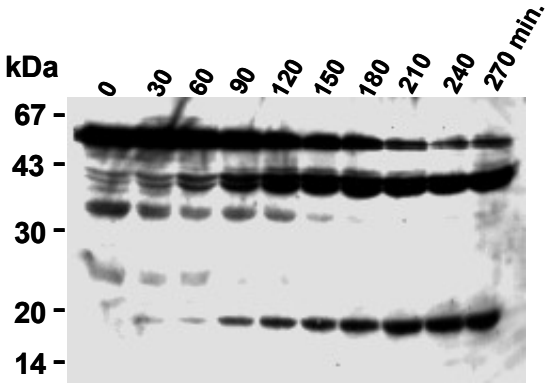
PROTOCOLS:

SDS-PAGE & Western Blotting

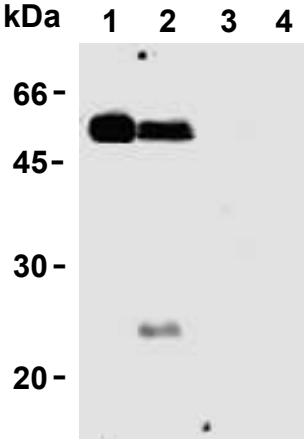
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge 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 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 (H+L chain) (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, HeLa, U937, MCF7, HEp-G2 and ZR-75-1)



Western blotting analysis of caspase-8 fragments in apoptosis induced Jurkat cells using M058-3.
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 caspase-8 expression in Jurkat (1), U937 (2), WR19L (3) and PC12 (4) using M058-3.

Apoptosis induction

1) 2x10^4 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) (Human) 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.

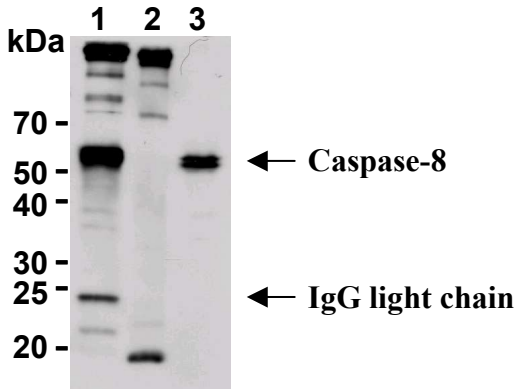
Immunoprecipitation

1) Collect the cultured cells from 75-cm^2 flask (containing about 1 x 10^7 cells).
2) Wash the cells twice with PBS and suspend with 800 µL of cold Lysis buffer [50 mM HEPES-KOH (pH 7.5), 250 mM NaCl, 0.1% NP-40, 5 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).
3) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
4) Add 50 µL of 50% protein A agarose beads in the supernatant. Incubate it at 4ºC with rotating for 60 minutes.
5) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C. Supernatant is equally divided into another two tubes.
6) Add Mouse IgG2b (isotype control) (MBL; code no. M077-3) or Anti-Caspase-8 (Human) mAb (M058-3) at the amount of as suggested in the **APPLICATIONS** to the supernatant. Vortex briefly and incubate with gently agitation for 60-120 minutes at 4°C.
7) Add 20 µL of 50% protein A agarose beads into the tube. Mix well and incubate with gentle

agitation for 30-60 minutes at 4°C.

8) Wash the beads 3-5 times with ice-cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
9) Resuspend the beads in 30 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 15 µL/lane for the SDS-PAGE analysis.
(See SDS-PAGE & Western blotting**.)**

(Positive control for Immunoprecipitation; Raji)



Immunoprecipitation of Caspase-8 from Raji with mouse IgG2b (2) or M058-3 (1) *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M058-3. Raji crude lysate was resolved in lane 3.*

RELATED PRODUCTS:

Please visit our web site at https://ruo.mbl.co.jp/.