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

MONOCLONAL ANTIBODY

Anti-Caspase-10 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M059-3	4C1	Mouse IgG1 κ	100 µL	1 mg/mL

BACKGROUND: Apoptosis is a major form of cell death characterized by several morphological features that include chromatin condensation and fragmentation, cell membrane blebbing, and formation of apoptotic bodies. These morphological changes occur via signaling pathway that leads to the recruitment and activation of caspases, a family of cysteine-containing, aspartate-specific proteases. Caspases exist as inactive proenzymes in cells and are activated through their processing into two subunits in response to apoptotic stimulation. Activated caspases cleave a variety of important cellular proteins, other caspases, and Bcl-2 family members, leading to a commitment to cell death. Caspase-10 (also known as Mch4, FLICE2 and ICE-LAP4) is a ~58 kDa protein. This caspase acts upstream of the apoptosis induced cascade. Once this caspase is activated by certain apoptotic stimuli, this protein may be responsible for the activation of the other caspases such as caspase-3, -4, -7, -8, and -9. This antibody was made against human-originated immunogen, and detects human caspase-10 specifically.

SOURCE: This antibody was purified from hybridoma (clone 4C1) 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 of human FLICE2.

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 detects specifically 57 and 58 kDa of human caspase-10, 43 kDa and 30 kDa of human active caspase-10 on Western blotting*. This antibody reacts with isoforms caspase-10/a, 10/b and 10/d**.
This reactivity is reported in the reference number 14)* and 23)**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat, HeLa, U937, HEp-G2	WR19L	Not tested
Reactivity on WB	+	-	

APPLICATIONS:
Western blotting; 1-10 µg/mL
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:
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REFERENCES:
1) Schleich, K., *et al., Cell Death Differ.* **23**, 681-694 (2016)
2) Estornes, Y., *et al., Cell Death Differ.* **19**, 1482-1494 (2012)
3) Cazanave, S. C., *et al., J. Biol. Chem.* **286**, 39336-39348 (2011)
4) Lafont, E., *et al., PLoS One* **5**, e13638 (2010)
5) Strauss, G., *et al., J. Exp. Med.* **206**, 1379-1393 (2009)
6) Verbrugge, I., *et al., Oncogene* **27**, 574-584 (2008)
7) Hyer, M. L., *et al., Cancer Res.* **68**, 2927-2933 (2008)
8) Koschny, R., *et al., Clin. Cancer Res.* **13**, 3403-3412 (2007)
9) Janssen, K., *et al., Blood* **110**, 3662-3672 (2007)
10) Milleron, R. S., and Bratton, S. B., *J. Biol. Chem.* **281**, 16991-17000 (2006)
11) Kurbanov, B. M., *et al., J. Invest. Dermatol.* **125**, 1010-1019 (2005)
12) Engels, I. H., *et al., Mol. Cell. Biol.* **25**, 2808-2818 (2005)
13) Milhas, D., *et al., J. Biol. Chem.* **280**, 19836-19842 (2005)
14) Park, S. J., *et al., J. Biol. Chem.* **279**, 51057-51067 (2004)
15) Kaiser, W. J. *et al., J. Immunol.* **172**, 1699-1710 (2004)
16) Ricci, M. S., *et al., Mol. Cell. Biol.* **24**, 8541-8555 (2004)
17) Wagner, K. W., *et al., J. Biol. Chem.* **279**, 35047-35052 (2004)
18) Nesterov, A., *et al., Cancer Res.* **64**, 3922-3927 (2004)
19) Wachter, T., *et al., J. Biol. Chem.* **279**, 52824-52834 (2004)
20) Strauss, G., *et al., J. Immunol.* **171**, 1172-1182 (2003)
21) Leverkus, M., *et al., Mol. Cell. Biol.* **23**, 777-790 (2003)
22) Franchi, L., *et al., Blood* **102**, 2910-2915 (2003)
23) Kischkel, F. C., *et al., J. Biol. Chem.* **276**, 46639-46646 (2001)
24) Cryns, V., *et al., Genes Dev.* **12**, 1551-1570 (1998)
25) Vincenz, C., *et al., J. Biol. Chem.* **272**, 6578-6583 (1997)
26) Nicholson, D.W., *et al., Trends Biochem. Sci.* **22**, 299-306 (1997)
27) Cohen, G.. M., *et al., Biochem. J.* **326**, 1-16 (1997)
28) Duan, H., *et al., J. Biol. Chem.* **271**, 1621-1625 (1996)
29) Arends, M. J., *et al., Int. Rev. Exp. Pathol.* **32**, 223-254 (1991)
Clone 4C1 is used in reference number 1)-23).

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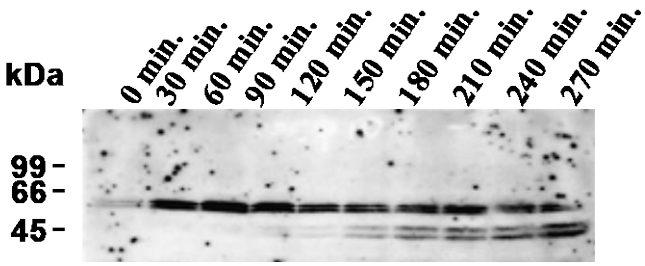
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.



Western blot analysis of Caspase-10 fragments expression in apoptosis induced Jurkat cells by anti-Fas monoclonal antibody (CH-11, MBL; code no. SY-001) using M059-3. M059-3 react with pro-caspase-10 and Intermediate form.

PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 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 protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (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 3 minutes and centrifuge. Load 10 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out 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 manufacturer'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 3 times).
9) Incubate the membrane with 1:5,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 5% 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) Drain excess buffer on the membrane, and incubate membrane with an 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 the membrane onto an X-ray film in a dark room for 2 minutes.
14) Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive controls for Western blotting; Jurkat, Raji, U937, HeLa and HEp-G2)

Apoptosis induction

1) 2 x 10^4 cells/50 µL of Jurkat cells or WR19L12a cells (human Fas transfectant) was 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 (MBL; code no. SY-001) diluted with RPMI 1640 containing 10% fetal calf serum.
3) Cultured for appropriate times at 37°C in 5% CO_2 incubator with RPMI 1640 containing 10% fetal calf serum.

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