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

MONOCLONAL ANTIBODY

Anti-Caspase-7 mAb

Code No.	Clone	Subclass	Quantity	Concentration
M053-3	4G2	Mouse IgG2b	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-7 (also known as Mch-3 / ICE-LAP3 / CMH-1) is a 35 kDa protein that has the highest similarity to caspase-3 (52% amino acid identity) between all caspase members. It has been involved in "effector" caspases (which include caspase 3, 6, 7) that are cleaved by "initiator" caspases (which include caspase 8, 9) into active form, and then, in turn cleave various cellular proteins for apoptosis. Recent study says that in Fas mediated hepatocyte apoptosis, active caspase-7 is associated almost exclusively with the mitochondrial and microsomal fractions, whereas active caspase-3 is confined primarily to the cytosol. It implies the different role of caspase-3 and -7 in the execution of apoptosis.

SOURCE: This antibody was purified from hybridoma (clone 4G2) 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 Caspase-7 protein corresponding to N-terminal amino acids (1-198 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, mouse and rat 35 kDa of pro-caspase-7 and cleaved 17 kDa product (large subunit) on Western blotting.
NOTE: Depend on the cell line or sample preparation, unidentified bands (~55 kDa and/or ~75 kDa) are occasionally observed. If necessary, please refer to other criteria to see how each result should be interpreted.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; 2 µg/200 µL of cell extract from 2.5 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat, U937, Raji	NIH/3T3, Ba/F3	Rat1
Reactivity on WB	+	+	+

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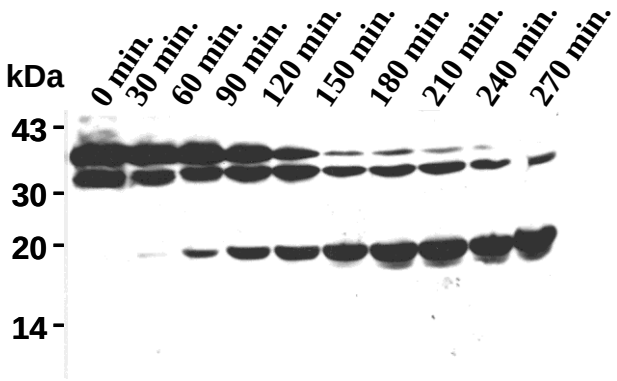

Western blot analysis of Caspase-7 fragments expression in apoptosis induced Jurkat cells by anti-Fas monoclonal antibody (CH-11, MBL; code no. SY-001) using M053-3. M053-3 react with pro-caspase-7, Intermediate form and active form.

REFERENCES:

1) Hitomi, J., *et al.*, *J. Cell Biol*. **165**, 347-356 (2004)
2) Chandler, J., *et al.*, *J. Biol. Chem*. **273**, 10815-10818 (1998)

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3) Cryns, V., *et al., Genes Dev.* **12**, 1551-1570 (1998)
4) Nicholson, D.W., *et al., Trends Biochem. Sci.* **22**, 299-306 (1997)
5) Cohen, G. M., *et al., Biochem. J.* **326**, 1-16 (1997)
6) Lippke, J. A., *et al., J. Biol.Chem.* **271**, 1825-1828 (1996)
7) Duan, H., *et al., J. Biol. Chem.* **271**, 1621-1625 (1996)
8) Fernandes-Alnemri, T., *et al., Cancer Res.* **55**, 6045-6052 (1995)
9) Arends, M. J., *et al., Int. Rev. Exp. Pathol.* **32**, 223-254 (1991)

Clone 4G2 is used in the reference number 1).

PROTOCOLS:

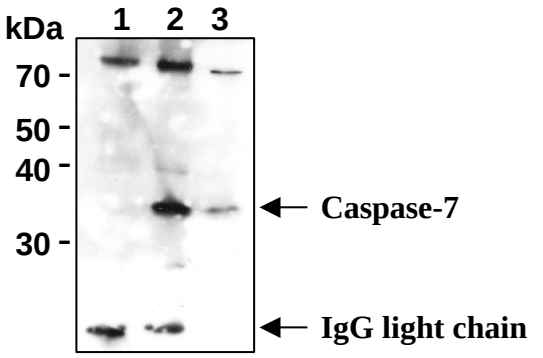
SDS-PAGE & Western Blotting

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 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% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 5% 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 5% 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 condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (10 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) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 2 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Jurkat, Raji, U937, NIH/3T3, Ba/F3 and Rat1)

Detection of cleaved Caspase-7 subunit

1) Prepare a 1 mM staurosporine stock solution by dissolving staurosporine (SIGMA) in DMSO.
2) Collect 1 x 10^7 of semi-confluently growing Jurkat cell by centrifugation, remove the medium and resuspend with 10 mL of growing medium containing 1 μM of staurosporine.
3) Incubate the cell suspension for 4 hours at 37°C. Harvest the cells by centrifugation.
4) Rinse the cells twice with PBS and resuspend in 1mL of Laemmli's SDS-PAGE sample buffer.
5) Lyse the cells by brief sonication (up to 10 sec) and boil for 5 minutes. Centrifuge it at 12,000 x g for one minute.
6) Use 5~20 μL/lane of the sample for the SDS-PAGE and Western blotting analysis.
(See **SDS-PAGE & Western blotting**.)



Immunoprecipitation of Caspase-7 from Raji with mouse IgG2b (1) or M053-3 (2) *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M053-3. Raji crude lysate was resolved in lane 3.*

Immunoprecipitation

1) Collect the cultured cells from 75-cm^2 flask (containing about 1 x 10^7 cells).
2) Wash the cells 2 times 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 4°C 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 tube.
6) Add Mouse IgG2b (isotype control) (MBL; code no. M077-3) or Anti-Caspase-7 mAb (M053-3) at the amount of as suggested in the **APPLICATIONS** to the supernatant. Vortex briefly and incubate with gentle 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)

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) (Human) 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.

RELATED PRODUCTS:

M073-3	Anti-Caspase-2 (Human) mAb (4F8)
M097-3	Anti-Caspase-3 (Human) mAb (1F3)
K0197-3	Anti-Caspase-3 (Human) mAb (AMI-3-1-11)
M087-3	Anti-Caspase-3 (Human) mAb (1F9)
M088-3	Anti-Caspase-3 (Human) mAb (7D12)
M029-3	Anti-Caspase-4 (Human) mAb (4B9)
M060-3	Anti-Caspase-5 mAb (4F7)
M070-3	Anti-Caspase-6 mAb (3E8)
M053-3	Anti-Caspase-7 mAb (4G2)
M032-3	Anti-Caspase-8 (Human) mAb (5F7)
M058-3	Anti-Caspase-8 (Human) mAb (5D3)
M054-3	Anti-Caspase-9 mAb (5B4)
M059-3	Anti-Caspase-10 (Human) mAb (4C1)
K0193-3	Anti-Caspase-14 (Human) mAb (8-1-71)
M028-3	Anti-TRAF1 (Mouse) mAb (3D4)
D038-3	Anti-Bcl-2 mAb (83-8B)
D038-5	Anti-Bcl-2 mAb-PE (83-8B)
M010-3	Anti-BAX (Human) mAb (4F11)
M033-3	Anti-FADD mAb (1F7)
M035-3	Anti-FADD (Human) mAb (4G3)
M037-3	Anti-DFF45 (ICAD) (Human) mAb (6B8)
M044-3	Anti-XIAP (MIHA/ILP-a) mAb (2F1)
M056-3	Anti-RAIDD (Human) mAb (4B12)
M072-3	Anti-BID (Human) mAb (5C9)
M074-3	Anti-Apaf-1 (Human) mAb (5C1)
M083-3	Anti-AcinusL mAb (3H8)
M112-3	Anti-TRAF2 mAb (6F8)
592	Anti-TRAF2 pAb (polyclonal)
597	Anti-TRAF6 (Mouse) pAb (polyclonal)
M092-3	Anti-TRAF6 (Mouse) mAb (1F8)
SY-001	Anti-Fas (CD95) (Human) mAb (CH-11)
D041-3	Anti-Fas Ligand (CD178) (Human) mAb (4H9)
D041-4	Anti-Fas Ligand (CD178) (Human) mAb-FITC (4H9)
D041-5	Anti-Fas Ligand (CD178) (Human) mAb-PE (4H9)
D041-6	Anti-Fas Ligand (CD178) (Human) mAb-Biotin (4H9)
D086-3	Anti-ASC (TMS1) (Human) mAb (23-4)
D132-3	Anti-CD279 (PD-1) (Human) mAb (J110)
D132-4	Anti-CD279 (PD-1) (Human) mAb-FITC (J110)
D133-3	Anti-CD279 (PD-1) (Human) mAb (J105)
D230-3	Anti-CD274 (PD-L1) (Human) mAb (27A2)
D161-3	Anti-MFG-E8 (Mouse) mAb (2422)
D199-3	Anti-MFG-E8 (Mouse) mAb (18A2-G10)
D184-3	Anti-Granulysin (Human) mAb (RB1)
D185-3	Anti-Granulysin (Human) mAb (RC8)
D185-6	Anti-Granulysin (Human) mAb-Biotin (RC8)
D186-3	Anti-Granulysin (Human) mAb (RF10)
D200-3	Anti-CD257 (BAFF/BLyS) (Human) mAb (1D6)
D200-4	Anti-CD257 (BAFF/BLyS) (Human) mAb-FITC (1D6)
D201-3	Anti-CD268 (BAFF-R/BR3) (Human) mAb (8A7)
D201-4	Anti-CD268 (BAFF-R/BR3) (Human) mAb-FITC (8A7)
D201-5	Anti-CD268 (BAFF-R/BR3) (Human) mAb-PE (8A7)
K0039-3	Anti-CD120a (TNF-R1) (Human) mAb (H398)
K0039-4	Anti-CD120a (TNF-R1) (Human) mAb-FITC (H398)
K0040-3	Anti-CD120b (TNF-R2) (Human) mAb (80M2)
K0040-4	Anti-CD120b (TNF-R2) (Human) mAb-FITC (80M2)
K0127-3	Anti-Daxx (Human) mAb (DAXX-01)
K0145-3	Anti-CD30 (Human) mAb (Ber-H2)
K0145-4	Anti-CD30 (Human) mAb-FITC0 (Ber-H2)
K0194-3	Anti-HtrA2 (Omi) (Human) mAb (18-1-83)
CM001-1	Anti-Cytochrome c mAb (1E4)
PM004	Anti-Smac (DIABLO) pAb (Polyclonal)
PD005	Anti-Vimentin Fragment (V1) pAb (Caspase-9 activation state antibody) (Polyclonal)
PD006	Anti-SETβ (p41/p42) pAb (polyclonal)
PD007	Anti-SETβ (p42) pAb (polyclonal)
PD008	Anti-SETβ (p41) pAb (polyclonal)
591	Anti-Bad pAb (polyclonal)
4700	MEBCYTO® Apoptosis Kit (Annexin V-FITC Kit)
8445	MEBSTAIN Apoptosis TUNEL Kit Direct
8442	MEBSTAIN Apoptosis TUNEL Kit III
4800	APOPCYTO™ Caspase-3 Colorimetric Assay Kit
4805	APOPCYTO™ Caspase-8 Colorimetric Assay Kit
4810	APOPCYTO™ Caspase-9 Colorimetric Assay Kit
4815	APOPCYTO™ Caspase-3 Fluorometric Assay Kit
4820	APOPCYTO™ Caspase-8 Fluorometric Assay Kit
4825	APOPCYTO™ Caspase-9 Fluorometric Assay Kit