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Not for use in diagnostic procedures.

MBL
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MONOCLONAL ANTIBODY

Anti-Caspase-9 mAb

Code No.	Clone	Subclass	Quantity	Concentration
M054-3	5B4	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-9, a 45 kDa protein (also known as ICE-LAP6 or Mch-6), is involved in one of relatively well characterized caspase cascades. It is triggered by cytochrome c released from mitochondria, which promotes the activation of caspase-9 by forming a complex with APAF-1 in the presence of dATP. For this activation, physical association of caspase-9 and APAF-1 in the complex is crucial and it is mediated by the interaction of respective caspase recruitment domain (CARD). This activated caspase-9 induces the downstream caspase-3 activation. It is also reported that the mice lacking caspase-9 die perinatally with a markedly enlarged and malformed cerebrum caused by reduced apoptosis during brain development, and that the thymocyte of the mice show resistance to a subset of apoptotic stimuli.

SOURCE: This antibody was purified from hybridoma (clone 5B4) 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 protein corresponding to N-terminal amino acids (1-250 aa) of human caspase-9.

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 45 kDa of pro-caspase-9 and cleaved 35 or 37 kDa products.

INTENDED USE:
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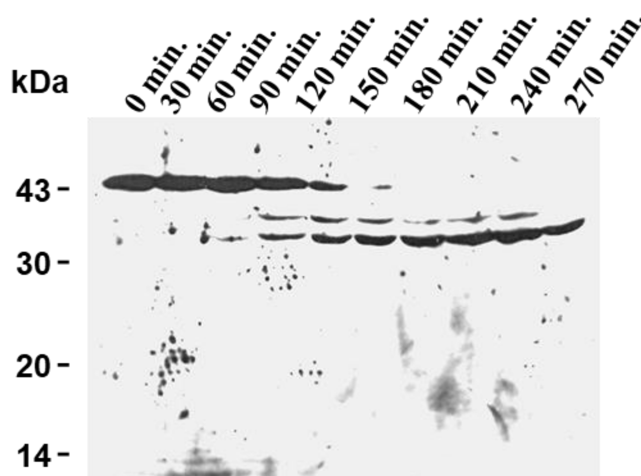
APPLICATIONS:
Western blotting; 1 µg/mL
Immunoprecipitation; Not recommended
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat, U937, Raji	NIH/3T3, Ba/F3	PC12
Reactivity on WB	+	+	+

REFERENCES:
Please visit our website at http://ruo.mbl.co.jp/.
As clone 5B4 is really famous all over the world, a lot of researches have been reported.



Western blotting analysis of Caspase-9 fragments expression in apoptosis induced Jurkat cells by anti-Fas monoclonal antibody (CH-11, MBL; code no. SY-001) using M054-3. M054-3 react with pro-caspase-9, Intermediate form and active form.

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% Methanol). 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 the 1:5,000 HRP-conjugated anti-mouse IgG (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, PC12)

Detection of Cleaved Caspase-9

1) Prepare a 1 mM staurosporine stock solution by dissolving staurosporine (SIGMA) in DMSO.
2) Collect 1 x 10^7 of semi-confluently grown Jurkat cells 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 1 mL of Laemmli's sample buffer.
5) Lyse the cells by brief sonication (up to 10 seconds) and boil for 5 minutes. Centrifuge it at 12000 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**.)

Induction of Apoptosis

1) 2 x 10^4 cells/50 µL of Jurkat cells or WR19L12a cells (human Fas transfectant) 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-human Fas monoclonal antibody (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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