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MBL
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MONOCLONAL ANTIBODY

Anti-Caspase-4 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M029-3	4B9	Mouse IgG1 κ	100 µL	1 mg/mL

BACKGROUND: The interleukin-1β converting enzyme (ICE)/CED-3 family proteases has been implicated in playing a fundamental role in programmed cell death. TX is a member of the ICE/CED-3 gene family encoding a cysteine protease that has a more than 50% sequence homology with ICE, especially in the region encoding the mature p20 and p10 ICE subunits and 30% sequence homology with Nedd-2/Ich-1L and CED-3. TX is able to cleave itself and the p30 ICE precursor and induces apoptosis in transfected cells1). TX is also a member of the caspase (CASP) family, CASP-4. An early biochemical event that occurs apoptosis in many cell types is the proteolytic cleavage of poly (ADP-ribose) polymerase (PARP), a nuclear enzyme involved in DNA repair. The several mammalian ICE homologues, ICE, TX, Nedd-2/Ich-1L and CPP32, are capable of cleaving PARP.

SOURCE: This antibody was purified from hybridoma (clone 4B9) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with recombinant protein corresponding to N-terminal amino acids (1-270 aa) of human TX.

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 caspase-4 (43 kDa) on Western blotting using total cell lysate from U937, HL60 and HUC-Fm (Human primary cultured fibroblast), and also reacts with 44 kDa of myc-tagged-TX expressed in 293T cell. Occasionally, unidentified 68 kDa band might be detected on western blotting in some cell lines.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	U937, HL60, HUC-Fm*, SK-N-SH, HeLa	Not tested	Not tested
Reactivity on WB	+		

*Human primary cultured fibroblast.

APPLICATIONS:

Western blotting; 1 µg/mL
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested*
*It is reported that this antibody can be used in this application in the following reference.
Flood, B., *et al.*, *Clin. Exp. Immunol.* **181**, 39-50 (2015)
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:

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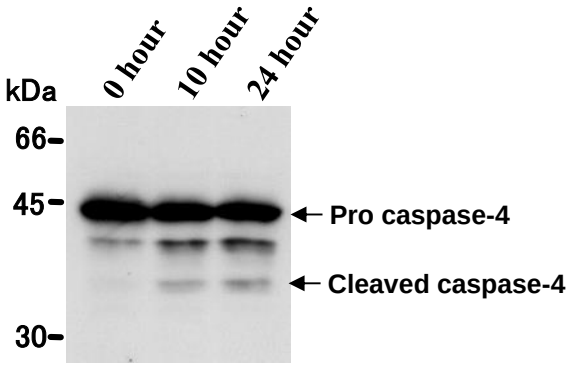

Western blotting analysis of Caspase-4 fragments expression in apoptosis induced SK-N-SH cells by 1 µg/mL tunicamycin using M029-3. M029-3 react with pro-caspase-4 and cleaved form.

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

PROTOCOL:

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 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 1% 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] (5 minutes x 3).
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 (10 minutes x 3).
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 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; U937, HL60, HUC-Fm, SK-N-SH and HeLa)

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