

MONOCLONAL ANTIBODY

Anti-Caspase-5

Code No.	Clone	Subclass	Quantity	Concentration
M060-3	4F7	Mouse IgG1 κ	100 μ g	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-5 (also known as ICE_{rel}-III or TY) is a ~47 kDa protein (418 aa), which is distinct members of the ICE/CED-3 family of cysteine proteases. This protein has 51% sequence identity with human interleukin-1- β -converting enzyme, 27% sequence identity with ICH-1L, 30% identity with human CPP32, and 24% sequence identity with the *C. elegans* CED-3 polypeptide. This ICE subfamily of caspase includes caspase-1, caspase-5, caspase-4, and caspase-13, and appears to play a primary role in cytokine maturation and inflammation. This antibody was made against human-originated immunogen, and detects human as well as mouse caspase-5.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma (clone 4F7) was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the recombinant C-terminal of caspase-5 (202-419 aa).

FORMULATION: 100 μ g IgG in 100 μ L 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 47 kDa of caspase-5 on Western blotting.

APPLICATIONS:

Western blotting; 1 μ g/mL for chemiluminescence detection system

Immunoprecipitation; Not tested

Immunohistochemistry; Not tested

Immunocytochemistry; Not tested

Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human
Cells	HeLa, U937, HEp-G2, HL-60
Reactivity on WB	+

*Cell lines such as Jurkat, Raji, and MCF7 were also tested and did not show reactivity on Western blotting.

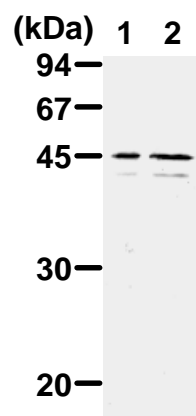
INTENDED USE:

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

REFERENCES:

- 1) Walsh, J. G., *et al.*, *J. Biol. Chem.* **286**, 32513-32524 (2011)
- 2) Eckhart, L., *et al.*, *Biochem. Biophys. Res. Commun.* **348**, 682-688 (2006)
- 3) Cryns, V., *et al.*, *Genes Dev.* **12**, 1551-1570 (1998)
- 4) Humke, E. W., *et al.*, *J. Biol. Chem.* **273**, 15702-15707 (1998)
- 5) Nicholson, D.W., *et al.*, *Trends Biochem. Sci.* **22**, 299-306 (1997)
- 6) Cohen, G.M., *et al.*, *Biochem. J.* **326**, 1-16 (1997)
- 7) Munday, N. A., *et al.*, *J. Biol. Chem.* **270**, 15870-15876 (1995)
- 8) Arends, M. J., *et al.*, *Int. Rev. Exp. Pathol.* **32**, 223-254 (1991)

This clone is used in the reference number 1) and 2).



Western blot analysis of Caspase-5

Lane 1: HL-60

Lane 2: HeLa

Immunoblotted with M060-3

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/cm² 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 PBS, pH 7.2 containing 5% skimmed milk as suggest 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 6 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; HL-60, HeLa)

- M058-3 Anti-Caspase-8 (5D3)
- M054-3 Anti-Caspase-9 (5B4)
- M059-3 Anti-Caspase-10 (4C1)
- K0206-3 Anti-Caspase-12 (14F7)
- K0207-3 Anti-Caspase-12 (14F4)
- K0193-3 Anti-Caspase-14 (8-1-71)

Kit

- 4690 APOPCYTO Annexin V-Azami-Green Apoptosis Detection Kit
- 4700 MEBCYTO Apoptosis Kit
- 8445 MEBSTAIN Apoptosis TUNEL Kit Direct
- 8441 MEBSTAIN Apoptosis TUNEL Kit II
- 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
- 4817 Intracellular Caspase-3 Activity Detection Kit
- 4822 Intracellular Caspase-8 Activity Detection Kit
- 4827 Intracellular Caspase-9 Activity Detection Kit
- 4830 APOPCYTO Intracellular Caspases Activity Detection Kit

Others

- E001 Active Caspase-3 (Human)
- E002 Active Caspase-7 (Human)
- 4800-510 Caspase-3 inhibitor Z-DEVD-FMK
- 4805-510 Caspase-8 inhibitor Z-DEVD-FMK
- 4810-510 Caspase-9 inhibitor Z-DEVD-FMK

RELATED PRODUCTS:

Antibodies

- M073-3 Anti-Caspase-2 (4F8)
- M097-3 Anti-Caspase-3 (1F3)
- K0197-3 Anti-Caspase-3 (AMI-3-1-11)
- M087-3 Anti-Caspase-3 (1F9)
- M088-3 Anti-Caspase-3 (7D12)
- M029-3 Anti-Caspase-4 (4B9)
- M060-3 Anti-Caspase-5 (4F7)
- M070-3 Anti-Caspase-6 (3E8)
- M053-3 Anti-Caspase-7 (4G2)
- M032-3 Anti-Caspase-8 (5F7)