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AM26609AF-N Monoclonal Antibody to Caspase-5 (202-419) - Azide Free

Alternate names:	CASP-5, CASP5, ICE(rel)-III, ICH-3 protease, ICH3, TY protease
Quantity:	0.1 mg
Concentration:	1.0 mg/ml
Background:	Apoptosis is a major form of cell death characterized by morphological features including chromatin condensation and fragmentation, cell membrane blebbing, and formation of apoptotic bodies. These morphological changes occur via signaling pathways that lead 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 commitm ent to cell death. Caspase-5 (also known as ICE rel -III or ICH-3) is a ~47 kDa member 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-1 L, 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.
Uniprot ID:	P51878
NCBI:	NP_001129581.1
GeneID:	838
Host / Isotype:	Mouse / IgG1
Recommended Isotype Controls:	SM20A (for use in rat samples)
Clone:	4F7
Immunogen:	Recombinant C-terminal region of caspase-5 (202-419 a.a.)
Format:	**State:** Liquid Ig fraction **Purification:** Protein A agarose **Buffer System:** PBS containing 50% glycerol, pH 7.2. Contains no preservatives.
Applications:	**Western blot:** 1 µg/ml for chemiluminescence detection system. For detatil s see protocol below. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Specificity:	This antibody reacts with 47 kDa of caspase-5.
Species Reactivity:	**Tested:** Human (HeLa, U937, HEp-G2, HL-60), Mouse (NIH/3T3, WR19L12A), Rat (PC12)
Add. Information:	This product was originally produced by MBL International.

Storage:

Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Shelf life: One year from despatch.

General Readings:

1. Cryns, V., et al., Genes Dev. 12, 1551-1570 (1998).
2. Humke, E. W., et al., J. Biol. Chem. 273, 15702-15707 (1998).
3. Nicholson, D.W., et al., Trends Biochem. Sci. 22, 299-306 (1997).
4. Cohen, G.M., et al., Biochem. J. 326, 1-16 (1997).
5. Munday, N. A ., et al., J. Biol. Chem. 270, 15870-15876 (1995).
6. Arends, M. J., et al., Int. Rev. Exp. Pathol. 32, 223-254 (1991).

Protocols:

SDS-PAGE & Western Blotting
1) Wash the cells 3 times with PBS and suspend with 10 volumes 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 proteas e inhibitors. Incubate it at 4 o 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 o C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make an 8 mg/mL solution.
3) Mix the sample with an equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 µ L 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 specific 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 o C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 5% skimmed milk as suggested in the APPLICATIONS for 1 hour at room temperature. (The optimal antibody concentration will depend on the experimental conditions.)
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 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 from the membrane, then incubate it with appropriate chemilumines cence reagents for 1 minute. Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
12) Expose to an X-ray film in a dark room for 1 minute. Develop the film as usual. The conditions for exposure and development may vary.
(Positive controls for Western blotting; HL-60, HeLa, NIH/3T3, PC12)

Pictures:

Western blot analysis of Caspase-5 expression in HL-60 (1), HeLa (2), NIH/3T3 (3) and PC12 (4) using AM26609AF-N.