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Product datasheet for AM26608AF-N

Caspase 10 (CASP10) (full length) Mouse Monoclonal Antibody [Clone ID: 4C1]

Product data:

Product Type:	Primary Antibodies
Clone Name:	4C1
Applications:	WB
Recommended Dilution:	**Western blot:** 1 µg/ml for chemiluminescence detection system. For details see protocol below.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Recombinant full-length of human FLICE2
Specificity:	This antibody detects specifically 57 and 58 kDa of human caspase-10, 43 kDa and 30 kDa of human active caspase-10 on Western blotting (Ref.8). This antibody reacts with isoforms caspase-10/a, 10/b and 10/d (Ref.17).
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	caspase 10
Database Link:	Entrez Gene 843 Human Q92851

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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. Ca spase-10 (also known as Mch4, FLICE2 and ICE-LAP4) is a ~58 kDa protein. This caspase acts upstream of the apoptosis induced cascade. Once this caspase is activated by certain apoptotic stimuli, this protein may be responsible for the activation of the other caspases such as caspase-3, -4, -7, -8, and -9. This antibody was made against human-originated immunogen, and detects human caspase-10 specifically.

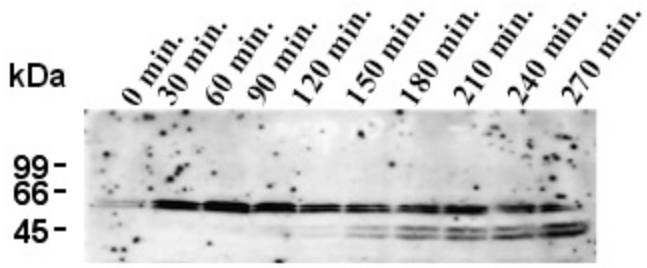
Synonyms: CASP-10, CASP10, MCH4, FLICE2

Note: This product was originally produced by MBL International.

Protocol:
SDS-PAGE & Western Blotting
1) Wash cells (approximately 1 x 10 7 cells) 3 times with PBS and resuspend them in 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 protease inhibitors at appropriate concentrations. Incubate it at 4 o C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (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 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 µ L of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out 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 manufactur er'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 o C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 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) Drain excess buffer on the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.

12) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
13) Expose the membrane onto an X-ray film in a dark room for 2 minutes.
14) Develop the film under usual settings. The conditions for exposure and development may vary.
(Positive controls for Western blotting; Jurkat, Raji, U937, HeLa, HEp-G2)
Apoptosis induction
1) 2 x 10 4 cells/50 µ L of Jurkat cells or WR19L12a cells (human Fas transfectant) was cultured in 96-well microplate at 37 o C in 5% CO 2 incubator with RPMI 1640 containing 10% fetal calf serum.
2) Add 50 µ L of 200 ng/mL anti-human Fas monoclonal antibody diluted with RPMI 1640 containing 10% fetal calf serum.
3) Cultured for appropriate times at 37 o C in 5% CO 2 incubator with RPMI 1640 containing 10% fetal calf serum.

Product images:



Western blot analysis of Caspase-10 fragments expression in apoptosis induced Jurkat cells by anti-Fas monoclonal antibody using AM26608AF-N. AM26608AF-N react with pro-caspase-10 and Intermediate form.