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Product datasheet for AM26602AF-N

Caspase-7 (CASP7) (1-198) Mouse Monoclonal Antibody [Clone ID: 4G2]

Product data:

Product Type:	Primary Antibodies
Clone Name:	4G2
Applications:	IP, WB
Recommended Dilution:	**Western blot:** 1 µg/ml for chemiluminescence detection system. **Immunoprecipitation:** 5 µg/300 µl of cell extract. For details see protocols below.
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	Recombinant Caspase-7 protein corresponding to N-terminal amino acids (1-198 a.a.)
Specificity:	This antibody reacts with 35 kDa of pro-caspase-7 and cleaved 17 kDa product (large subunit) on Western blotting. May also detect ~30 kDa intermediate form. NOTE: Depending on the cell line or sample preparation, unidentified bands (~55 kDa and/or ~75 kDa) are occasionally observed. If necessary, please refer to other criteria to see how each result should be interpreted.
Formulation:	PBS containing 50% glycerol, pH 7.2. Contains no preservatives. 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 7
Database Link:	Entrez Gene 840 Human P55210

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Background: Apoptosis is a major form of cell death characterized by morpho logical features including chromatin condensation and fragmentation, cell membrane blebbing, and formation of apoptotic bodies. These morphological changes occur vi a signaling pathways that lead to the recruitment and activation of caspases, a family of cysteine-containing, as partate-specific proteases. Caspases exist as inactive pr oenzymes 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. Casp ase-7 (also known as Mch-3 / ICE-LAP3 / CMH-1) is a 35 kDa protein that has the highest similarity to caspase-3 (52% amino acid identity) among all caspase members. It has been identified as one of the ''effector'' caspases (which include caspase 3, 6, 7) that are cleaved by ''initiator'' caspases (which include caspase 8, 9) into active form, and then, in turn, cleave various cellular proteins for apoptosis. Recent study says that in Fas-mediated hepatocyte apoptosis, active caspase-7 is associated almost exclusivel y with the mitochondrial and microsomal fractions, whereas active caspase-3 is confined primarily to the cytosol. It implies a different role of caspase-3 and -7 in the execution of apoptosis.

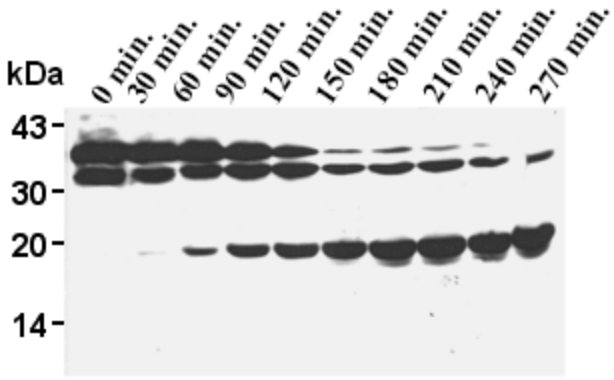
Synonyms: CASP-7, CASP7, MCH3, CMH-1

Note: This product was originally produced by MBL International.

Protocol:
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 protease 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 (10 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 (10 minutes x 3 times).
11) Wipe excess buffer from the membrane, then incubate it with appropriate chemilum inescence 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; Jurkat, Raji, U937, NIH/3T3, Ba/F3, Rat1
Immunoprecipitation
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 protease 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.
3) Add primary antibody as suggested in the APPLICATIONS into 300 µ L of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4 o C. Add 20 µ L of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4 o C
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 µ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µ L/lane for the SDS-PAGE analysis. (See SDS-PAGE & Western blotting.)
Detection of cleaved Caspase 7 subunit
1) Prepare a 1mM staurosporine stock solution by dissolving staurosporine in DMSO.
2) Collect 1 x 10 7 of semi-confluently growing Jurkat cell by centrifugation, remove the medium and resuspend with 10 mL of growing medium containing 1 µ M 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 1mL of Laemmli SDS-PAGE sample buffer.
5) Lyse the cells by brief sonication (up to 10 sec) and boil for 5min. Centrifuge it at 12000 x g for one minute.
6) Use 5~20 µ L/lane of the sample for the SDS-PAGE and Western blotting analysis (See SDS-PAGE & Western blotting.)
Apoptosis induction
1) 2x10 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-7 fragments expression in apoptosis induced Jurkat cells by anti-Fas monoclonal antibody using AM26602AF-N. AM26602AF-N react with pro-caspase-7, Intermediate form and active form.