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Product datasheet for AM26585AF-N

Caspase 4 (CASP4) (1-270) Mouse Monoclonal Antibody [Clone ID: 4B9]

Product data:

Product Type:	Primary Antibodies
Clone Name:	4B9
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 protein corresponding to N-terminal amino acids (1-270 aa) of human TX
Specificity:	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, uni dentified 68 kDa band might be detected on western blotting in some cell lines.
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 4
Database Link:	Entrez Gene 837 Human P49662

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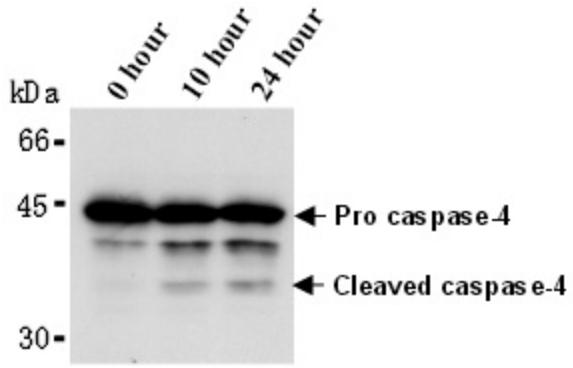
Background: The interleukin-1 β converting enzyme (ICE)/CED-3 family pr oteases has been implicated in playing a fundamental role in programmed cell death. TX is a member of the ICE/ CED-3 gene family encoding a cysteine protease that ha s a more than 50% sequence homology with ICE, especially in the 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 IC E 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 in volved in DNA repair. The several mammalian ICE homologues, ICE, TX, Nedd-2/Ich-1L and CPP32, ar e capable of cleaving PARP.

Synonyms: CASP4, ICH2, ICE(rel)-II, Protease ICH-2, Protease TX

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 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 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 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 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 manufact ure'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 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 3 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG 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 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 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, HeLa)

Product images:



Western blot analysis of Caspase-4 fragments expression in apoptosis induced SK-N-SH cells by 1 µg/mL tunicamycin using AM26585AF-N. AM26585AF-N reacts with pro-caspase-4 and cleaved form.