Datasheet for ABIN5541751

anti-Caspase 4 antibody (N-Term)

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Overview

Quantity:	0.1 mg
Target:	Caspase 4 (CASP4)
Binding Specificity:	AA 1-270, N-Term
Reactivity:	Human
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This Caspase 4 antibody is un-conjugated
Application:	Western Blotting (WB)

Product Details

Immunogen:	Recombinant protein corresponding to N-terminal amino acids (1-270 aa) of human TX
Clone:	4B9
Isotype:	IgG1
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.
Purification:	Protein A agarose

Target Details

Target:	Caspase 4 (CASP4)

Target Details

Alternative Name:	caspase-4 (CASP4 Products)
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.
UniProt:	P49662
Pathways:	Apoptosis, Caspase Cascade in Apoptosis, Positive Regulation of Endopeptidase Activity

Application Details

Application Notes:	Western blot: 1 µg/mL for chemiluminescence detection system. For details see protocol below.
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 %

Application Details

	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)
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	PBS containing 50 % glycerol, pH 7.2. No preservative is contained.
Preservative:	Azide free
Storage:	-20 °C
Storage Comment:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing. Shelf life: One year from despatch.