OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for AM26607AF-N

Caspase 8 (CASP8) (180-480) Mouse Monoclonal Antibody [Clone ID: 5D3]

Product data:

Product Type:	Primary Antibodies
Clone Name:	5D3
Applications:	IP, WB
Recommended Dilution:	**Western blot:** 1 µg/ml for chemiluminescence detection system. **Immunoprecipitation:** 2 µg/500 µl of cell extract. For details see protocol below.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	Recombinant human caspase s-8 corresponding to C-terminal amino acids (180-480 aa)
Specificity:	This antibody reacts with caspase-8.
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 8
Database Link:	Entrez Gene 841 Human Q14790

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Background: Caspase-8 (FLICE/MACH/Mch5) is a member of the ICE (interleukin- 1 converting enzyme)/CED-3 family cysteine protease. It is the most upstream protease that recei ves the activation signal from the Fas (APO1/CD95) and TNFR1 (Tumor Necrosis Factor Receptor 1) to initiate the ap optotic protease cascade that leads to activation of ICE/CED-3 family proteases. Caspase-8 has high homologous region to the ICE/CED-3 family in C-terminal and two death effecter domains (DED) in N-terminal. Binding of caspase-8 to FADD (MORT1) through association of their DED, and consequent activation of the caspases by th eir proteolytic cleavage, are thought to be critical steps in the initiation of Fas- and TNFR1-induced apoptosis. Recen tly the inhibitor of Fas- and TNFR1-induced apoptosis is identified, called I-FLICE (FLIP/Casper/FLAME/CASH). I-FLICE has high homology to caspase-8 and it contains two DED, which interacts with caspase-8 and FADD, and potently inhibits Fas- and TNFR1-induced apoptosis.

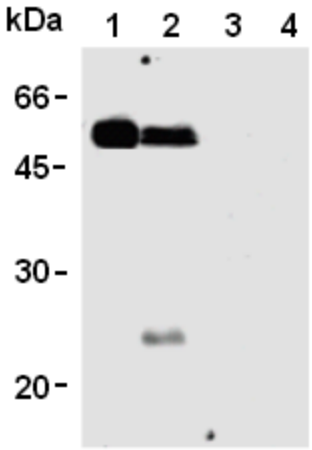
Synonyms: CASP-8, CASP8, MCH5, CAP4

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 2 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 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 6 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 (5 minutes x 6 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemilumin escence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.

12) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.
(Positive controls for Western blotting; Jurkat, Raji, HeLa, U937, MCF7, HEp-G2, ZR-75-1)
Immunoprecipitation
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.
3) Add primary antibody as suggest in the APPLICATIONS into 500 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.)

Product images:



Western blot analysis of caspase-8 expression in Jurkat (1), U937 (2), WR19L (3) and PC12 (4) using AM26607AF-N.