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Product datasheet for AM26588AF-N

Caspase 8 (CASP8) (176-460) Mouse Monoclonal Antibody [Clone ID: 5F7]

Product data:

Product Type:	Primary Antibodies
Clone Name:	5F7
Applications:	WB
Recommended Dilution:	**Western blot:** 1:1000 for chemiluminescence detection system. For details see protocol below.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	GST-FLICE fusion protein corresponding to C-terminal amino acids of human FLICE (176-460 a.a.)
Specificity:	This antibody reacts with caspase 8 on Western blotting. It doesn't cross-react with other caspases including caspase10 (FLICE2) which has high homology to caspase 8, however cross-reactivity with I-FLICE has not been examined. The antibody detects 55 KD of human caspase 8a (MACH α 1) as well as 54KD of human caspase 8b (MACH α 2) on Western blotting with total cell lysate from Jurkat, Raji, U937 and HeLa. Also detects the 18 kDa active form, and the 43 and 26 kDa intermediate forms. May detect a n unidentiffied 72 kDa band in some cell lines.
Formulation:	Protein-A Sepharose, PBS containing 50% glycerol. Contains no preservatives. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
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 β converting enzyme)/CED-3 family cysteine protease. It is the most upstream protease that receives the activation signal from the Fas (APO1/CD95) and TNFR1 (Tumor Necrosis Factor Receptor 1) to initiate the apoptotic protease cascade that leads to activation of ICE/CED-3 family proteases. Caspase 8 has high homologous reagion 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 their proteolytic cleavage, are thought to be critical steps in the initiation of Fas- and TNFR1-induced apoptosis 1) 2) 3) . Recently 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 4) and FADD 5) , 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) Boil all samples for 3~5 minutes. Load 1 0 µ l of cell lysate or tissue homogenate (5~20 µ g total protein) to each well of an SDS-polyacrylamide gel and electrophorese in a 1 mm thick gel.
2) Transfer to a polyvinylidene difluoride (PVDF) membrane at 10V for 1hour in a semi-dry transfer system. (Transfer Buffer: 25mM Tris, 190mM glycine, 20% MeOH).
3) The transferred proteins can be visualized by staining the membrane for 1 minute with Ponceau S. Rinse the membrane with PBS.
4) Non-specific binding sites are blocked by immersing the membrane in 5% Skim Milk / PBS / 0.05% Tween20 for 1 hour at room temperature, or for overnight at 4 C.
5) Incubate in primary antibody diluted as suggest ed in the APPLICATIONS for 1 hour at room temperature. (The optimal antibody concentration will depend on the experimental variables and the abundance of the antigen.)
6) Wash the membrane 3 times with PBS, 0.05% Tween20 for 5~10 minutes per wash.
7) Incubate in Horseradish Peroxidase conjugated goat anti-mouse diluted 1:3000 in PBS, 0.05% Tween20 for 45 minutes at room temperature.
8) Wash the membrane 3 times with PBS, 0.05% Tween20 for 10 minutes per wash.
9) Incubate in Amersham ECL Reagent for 1 minute. Drain membrane, remove excess ECL Reagent by dabbing with a Kimwipe, and seal in plastic wrap.
10) Expose to ECL hyperfilm in a dark room for 30 seconds. Develop as usual for autoradiogram or X-ray. The condition s for development and exposure may vary.