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Product Information

Caspase 8, Human
Recombinant, expressed in *E. coli*

Catalog Number **C1099**
Storage Temperature –70 °C

EC 3.4.22.61
Synonyms: Mach, FLICE, Mch5

Product Description
Caspase-8 (Mach, FLICE, Mch5)[1-3] is a member of the caspase family of cysteine proteases that play an essential role in the execution phase of apoptosis. These enzymes share a dominant primary specificity for cleaving bonds following aspartic acid residues. "Initiator" caspases (such as caspase-8) activate "effector" caspases (e.g., caspase-3 and caspase-7). The effector caspases then cleave cellular substrates, ultimately leading to the morphological changes of apoptosis.[4-6]

Caspases are synthesized as inactive pro-enzymes. The precursor proteins contain N-terminal pro-sequences of various lengths followed by the p20 and p10 subunits. Caspases are activated by cleavage at specific Asp residues to produce two subunits of approximately 20 kDa (p20) and 10 kDa (p10), which together form the heterodimeric active protease.[5,6] In some cases, these subunits are separated by a linker that may be involved in regulation of the activation of the caspase. All caspases contain an active-site pentapeptide of general structure QACXG (where X is R, Q or G). The amino acids Cys-285 and His-237 involved in catalysis, and those involved in forming the P1 carboxylate binding pocket (Arg-179, Gln-283, Arg-341 and Ser-347) are conserved in all caspases, except for the substitution of Thr for Ser-347 in caspase-8. This explains the absolute requirement for an Asp in the P1 position. Residues forming the P2–P4 binding pocket are not well conserved. This suggests they may determine the substrate specificities of the caspases. Evidence suggests that not all caspases are required for cell death, and some caspases appear to be more important than others.[5]

Caspase-8 is at the apex of the apoptotic pathway that links death signals to caspase activation. At least eight different caspase-8 isoforms (designated as caspase-8a-h also termed FLICE/MACHα1, MACHα2, MACHα3, MACHβ1-4 and MCH5) have been described at the mRNA level.[7]

The predominant caspase-8 isoforms expressed in cells, caspase 8a/b (479 and 464 aa), are formed as inactive 55 kDa and 54 kDa precursors, respectively. The N-terminal region of caspase-8 contains two death effector domains (DEDs), which facilitate the interaction with the adaptor protein FADD/MORT-1. The recruitment and activation of caspase-8 is inhibited by v-FLIPs and I-FLICE proteins.[8] Upon activation of Fas or TNFR-1, caspase-8 is recruited to FADD on the receptor complex to initiate the caspase cascade and induce apoptosis.[1,2]

Caspase-8 is activated by oligomerization-induced processing, resulting in removal of its death domain and cleavage into active large and small subunits which are released in the cytosol.[9] These subunits form a proteolytically active heterodimer that is capable of cleaving other downstream caspase family members such as caspase-3, -6, and -7.[5,10-12] These, in turn, cleave various cellular substrates such as PARP and DFF, leading eventually to the apoptotic death of cells.

This recombinant human caspase-8 with a C-terminal histidine tag was expressed in *E. coli* as truncated pro-caspase-8 (amino acids 213-496) and purified. It is the fully active protein, consisting of 18 kDa large and 12.4 kDa small subunits. The small subunit contains the histidine tag.

The product is supplied as a solution in 10% sucrose containing 20 mM Tris, pH 8.0, 500 mM NaCl, 20 mM 2-mercaptoethanol, 2.5 mM EDTA, 0.1% CHAPS, and 150 mM imidazole.

Purity: ≥90% (SDS-PAGE)

Activity: ≥1,000 units/mg protein

Unit Definition: One unit will hydrolyze one nmol of Ac-Ile-Glu-Thr-Asp-pNA per minute at pH 7.5 at 25 °C.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
Store at −70 °C. Repeated freezing and thawing is not recommended. Storage in 'frost-free' freezers is not recommended.

References

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