

Product Information

MONOCLONAL ANTI-CASPASE 8 CLONE CAS8

Purified Mouse Immunoglobulin

Product Number **C 4106**

Product Description

Monoclonal Anti-Caspase 8 (mouse IgM isotype) is derived from the CAS8 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with a recombinant human caspase 8. The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-Caspase 8 reacts specifically with human caspase 8. The antibody may be used for ELISA and immunoblotting (53 kDa). Additional bands of 33 kDa and 10 kDa may be seen in certain preparations.

Apoptosis, an evolutionary conserved form of cell suicide, requires specialized machinery. The central component of this machinery is a proteolytic system involving a family of proteases called caspases. These enzymes participate in a cascade that is triggered in response to proapoptotic signals and culminates in cleavage of a set of proteins, resulting in disassembly of the cell.

Caspases (**C**ysteine-requiring **A**spartate protease) are a family of proteases that share similarities in amino acid sequences, structure, and substrate specificity.¹⁻⁴ Caspases can be grouped into three subfamilies based on their amino acid sequence homology. The caspase 1 (ICE-type caspases) subfamily contains caspases 1, 4, 5, 11, and 13. This subfamily along with caspase 12, has a role in inflammation as well as in apoptosis; these proteases may also be indirectly involved in apoptosis as activators of other caspases (upstream activity). Caspase-8 and -10 are involved in death receptor mediated apoptosis. The caspase 2 subfamily contains caspases 2 and 9, while the caspase 3 subfamily contains caspases 3, 6 and 7, and are effectors of apoptosis (downstream activity).

Caspases are normally present in the cell as inactive procaspases. The proenzymes (30-50 kDa) contain three domains: an NH₂-terminal prodomain, a large subunit (17-22 kDa), and a small subunit (10-12 kDa). Proteolytic cleavage at Asp residues removes the regulatory N-terminal prodomain and cleaves the proenzyme into the large and small subunits.

The subunits self-associate into heterodimers that in turn form the active caspase, a tetramer consisting of two large and two small subunits. The active caspases continue the cascade by autocleaving, cleaving other procaspases, or cleaving other key proteins such as (but not limited to) poly(ADP-ribose) polymerase (PARP), DNA-dependent protein kinase (DNA-PK), lamins, nuclear mitotic apparatus protein (NuMA), and sterol regulatory element binding proteins (SREBPs).

The caspase 8 (also known as MACH, FLICE and Mch5) gene encodes protein products of 9.5-57.7 kDa. Eight isoforms of caspase 8 (caspase 8/a-h), including FLICE (CAP4/MACH α 1), MACH α 2 and MACH α 3, MACH β and Mch5 have been described at the mRNA level. However, only two caspase 8 isoforms (8/a and 8/b, 55.7 and 53.7 kDa, respectively) are detected in significant amounts at the protein level.⁵ Caspase 8 undergoes a cleavage upon activation (leading to p26/p24/p18/p10 subunits, for isoforms 8/a and 8/b).⁵ It is the initiating caspase in the apoptotic cascade that is activated by engagement to death receptors belonging to the tumor necrosis factor receptor family. Caspase 8 contains two NH₂-terminal tandem repeats within the prodomain, which are homologous to the death effector domain (DED) in the adaptor molecule FADD and allow for its recruitment to the receptor signaling complex. The remainder of the molecule is highly similar to the CED-3 subfamily of caspases. Caspase 8 has been shown to process a variety of proapoptotic caspases; but it did not activate members of the ICE subfamily, except of ERICE (caspase 13).⁶ Monoclonal antibodies reacting specifically with caspase 8 are a useful tool for the study of the protease network involved in development and regulation, governing the life and death of cells and tissues.

Reagent

Monoclonal Anti-Caspase 8 is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody Concentration: Approx. 2 mg/ml.

Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution.

Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2 °C to 8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

A working concentration of 5-10 µg/ml is determined by immunoblotting using a whole extract of a HeLa cells mitochondrial preparation.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

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2. Cohen, G.M., *Biochem. J.*, **326**, 1-16 (1997).
3. Cryns, V., and Yuan, J., *Genes Develop.*, **12**, 1551-1570 (1998).
4. Kidd, V.J., *Annu. Rev. Physiol.*, **60**, 533-573 (1998).
5. Scaffidi, C., et al., *J. Biol. Chem.*, **272**, 26953-26958 (1997).
6. Humke, E.W., et al., *J. Biol. Chem.*, **273**, 15702-15707 (1998).

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