

## Product Information

### MONOCLONAL ANTI-CASPASE-5

#### CLONE CAS5

Purified Mouse Immunoglobulin

Product Number **C 6979**

#### Product Description

Monoclonal Anti-Caspase-5 (mouse IgG2b isotype) is derived from the CAS5 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with a synthetic peptide corresponding to amino acids 300-311 of human caspase-5 protein, conjugated to KLH. 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-5 reacts specifically with the "procaspase" form of caspase-5 (also known as Ich-3, ICERel-III, and TY). The epitope recognized by the antibody resides within amino acids 300-311 of human caspase-5 molecule. The antibody may be used for ELISA and immunoblotting (a doublet at approx. 42 kDa, with additional bands at approx. 75 kDa). Reactivity has been observed with human caspase-5.

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.<sup>1</sup> 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 greater role in inflammation than in apoptosis; these proteases may also be indirectly involved in apoptosis as activators of other caspases (upstream activity). The caspase 2 subfamily contains caspases 2 and 9, while the caspase 3 subfamily contains

caspases 3, 6, 7, 8 and 10, 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 NH<sub>2</sub>-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 as 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).

Caspase 5 (also known as Ich-3, ICERel-III, and TY) is a 418 amino acids protein (42 kDa, 47 kDa according to some reports) that belongs to the Ced3/ICE family of cystein proteases. Caspase 5 is an "upstream" caspase with a long prodomain. It undergoes a cleavage upon activation, leading to p20 and p10 subunits which have high homology with several other caspases over the equivalent coding sequences.<sup>2-6</sup> Monoclonal antibodies reacting specifically with caspase-5 are 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-5 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 hazardous 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 HeLa cells activated with lipopolysaccharide (LPS).

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

**References**

1. Thornberry, N.A., and Lazebnik, Y., *Science*, **281**, 1312-1316 (1998).
2. Wang, L., et al., *Cell*, **78**, 739-750 (1995).
3. Kamens, J., et al., *J. Biol. Chem.*, **270**, 15250-15256 (1995).
4. Fernandes-Alnemri, T., et al., *Cancer Res.*, **55**, 2737-2742 (1997).
5. Cohen, G.M., *Biochem. J.*, **326**, 1-16 (1997).
6. Munday, N.A., et al., *J. Biol. Chem.*, **270**, 15870-15876 (1995).

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