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ProductInformation

Anti-Calpastatin, N-Terminal (Domain-L)
Testis Isoform
Developed in Rabbit
Affinity Isolated Antibody

Product Number **C 8238**

Product Description

Anti-Calpastatin, N-Terminal (Domain-L) Testis Isoform is developed in rabbit using a synthetic peptide corresponding to the amino terminal end of the testis isoform of human calpastatin as immunogen. Affinity isolated antigen specific antibody is obtained from rabbit anti-calpastatin antiserum by immuno-specific purification which removes essentially all rabbit serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Anti-Calpastatin, N-Terminal (Domain-L) Testis Isoform may be used for the detection and localization of human, mouse, and rat calpastatin. By immunoblotting against the reduced protein, the antibody recognizes bands at 70 kDa, 52 kDa, and a series of further cleaved active forms in cell lysates. The antibody will also bind to the non-reduced protein, and thus has application for other immunochemical techniques, such as immunoprecipitation, immunohistochemistry, and ELISA.

Calpastatin is the endogenous intracellular inhibitor of calpains (calcium-dependent cysteine proteases) and expressed in virtually all cell types. It is produced several fold in excess of the calpain concentration. Fourteen distinct calpains have been found in humans and rodents with two different small subunit accessory proteins. By far the most studied are calpains 1 and 2, the ubiquitous calpains that differ in their sensitivity to calcium. Calpastatin inhibits calpain 1 and 2, but does not appear to inhibit calpain 3 (although there are conflicting reports in the literature). The other calpain family members have more limited tissue distribution and perhaps different functions. Little is known about the efficacy of calpastatin on these isoforms.

A single gene for calpastatin is alternatively spliced into many isoforms of calpastatin with different exons matched together and differentially expressed in various tissues and cell lines.[1] Full-length calpastatin encodes a 708 amino acid protein with a predicted mass of 76.5 kDa, but due to modifications and cysteine-rich regions, the protein migrates at 110 kDa on reduced SDS PAGE. Full-length calpastatin contains an aminoterminal “L” domain, followed by 4 repeats of the inhibitory domains. Other splice variants include the L domain with 1, 2, or 3 inhibitory domains. The inhibitory domains are also expressed without the L domain. The inhibitory domains are further divided into subdomains A, B, and C. Subdomains A and C are thought to enhance binding, and subdomain B contains inhibitory activity.[2] Post-translational modifications include phosphorylation, which modulates membrane binding. Calpastatin is also readily cleaved separating the L domain from the inhibitory domain and then further degraded to abolish activity.

Sera from rheumatoid arthritis patients often contains inhibitory autoantibodies to calpastatin, which effectively increases the calpain activity.[3] Auto-antibodies to calpastatin have also been detected in infertile males, where it seems to compromise sperm activity.[4]

Reagent

Anti-Calpastatin, N-Terminal (Domain-L) Testis Isoform is supplied in phosphate buffered saline containing 50% glycerol and 0.05% sodium azide. The protein concentration is approximately 1 mg/ml.

Precautions and Disclaimer

Due to the sodium azide content a material safety data 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 2-8 °C for up to six months. For extended storage, the solution may be aliquoted and stored at –20 °C. Do not store below –22 °C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile
By immunoblotting, a minimum working antibody dilution of 1:1,000 is recommended using an alkaline phosphatase conjugated secondary antibody and BCIP/NBT as the substrate. A starting antibody dilution of 1:5,000 is recommended for chemiluminescent substrates. Cell lysates work well for immunoblotting

Note: Higher antibody dilutions may be necessary for non-human samples.

In order to obtain the best results and assay sensitivity in various techniques and preparations, we recommend determining the optimum working dilution by titration.

References

1. Woon, J.L., et al., Molecular diversity in amino-terminal domains of human calpastatin by exon skipping. J. Biol. Chem., **267**, 8437-8442 (1992).
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3. Despres, N., et al., Detection and expression of a cDNA clone that encodes a polypeptide containing two inhibitory domains of human calpastatin and its recognition by rheumatoid arthritis sera. J. Clin. Invest., **95**, 1891-1896 (1995).
4. Li, S., et al., Molecular cloning and characterization of functional domains of a human testis-specific isoform of calpastatin. Biol. Reprod., **63**, 172-178 (2000).

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