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ProductInformation

Anti-Calpain-9 (Domain II, Catalytic Domain), Large Subunit
Developed in Rabbit
Affinity Isolated Antibody

Product Number **C 2239**

Product Description

Anti-Calpain-9 (Domain II, Catalytic Domain), Large Subunit is developed in rabbit using a synthetic peptide corresponding to the catalytic domain II of the large subunit of human calpain 9 (capn-9, nCL-4, GC36) as immunogen. The antibody is affinity purified using agarose to which the immunogen peptide has been bound.

Anti-Calpain-9 (Domain II, Catalytic Domain), Large Subunit recognizes human, rat, and mouse calpain 9 by various immunochemical techniques including immunoblotting, immunoprecipitation, immunohistochemistry, and ELISA. The antibody recognizes the latent and active (aminotruncated) protein. It does not crossreact with other calpain family members (calpain 1, calpain 2, calpain 3, LP-82/85 calpain, nCL-2, nCL-3, etc.). The antibody binds to the reduced and non-reduced protein. By immunoblotting against the reduced protein, the antibody reacts with bands at approximately 79 kDa, 68 kDa, 48 kDa, and a series of smaller forms.

Calpains are calcium-activated, non-lysosomal cysteine proteases that cleave cytoskeletal and submembranous proteins. The calpains have papain-like activity, thus the –pain nomenclature. The calpain (calcium-dependent proteinase or calcium activated neutral protease) system consists of two ubiquitous forms of calpain (calpain 1 and calpain 2), a series of tissue specific calpains (calpains 3-15), and a calpain inhibitory protein (calpastatin). The calpain system plays a regulatory role in cellular protein metabolism.[1] This regulatory role may have important implications in platelet aggregation and pathologies associated with altered calcium homeostasis and protein metabolism such as ischemic cell injury and degenerative diseases. Inhibitors of calpain have been shown to block dexamethasone- and low-level irradiation-induced apoptosis in thymocytes suggesting that calpain has a regulatory or mechanistic role in apoptotic cell death.

The “classical” calpain family members (calpain 1 and calpain 2) are heterodimers and consist of a common regulatory small subunit (calpain-S1), and a large variable catalytic subunit. Domains in the large subunit include the aminoterminal domain I, the proteinase domain II, domain III, and EF-hand (Ca^{2+}-binding) domain-IV.[1] Calpain 9, also known as nCL-9 (novel calpain-9) and GC36 (gastric cancer gene #36), is an intracellular calcium-sensitive cysteine protease. First isolated in a search for stomach-specific calpains,[2] calpain 9 was shown to be in highest abundance in stomach and intestinal tract, similar to calpain 8. Calpain 9 appears to have a wider distribution than initially thought. Later, calpain 9 was identified as GC36, a gene down-regulated in gastric cancer.[3] One of the calpain 9 sequences has a deletion near the end of the catalytic domain, perhaps affecting the enzymatic function. Like the classical calpains (calpain-1 and calpain-2), calpain 9 contains EF-hand calcium binding domains, and likely requires calcium for activity. The latent large subunit is 79 kDa, and aminoterminal truncations at activation yields approximately 68 kDa isoforms. Also, a cascade of smaller forms can be seen with further activation. Recombinant calpain 9, expressed in baculovirus,[4] requires the calpain-S1 subunit to fold properly, and forms a heterodimer with the S1 subunit. The recombinant calpain quickly degrades once calcium is introduced at *in vivo* temperatures. Antisense reduction in calpain 9 levels leads to neoplastic transformation in NIH3T3 cells suggesting important roles for the enzyme in normal cells.[5]

Calpain 9, like calpain 1 and calpain 2, is ubiquitously expressed. Calpains are present in all mammalian tissues and are involved in a variety of processes including cytoskeletal reorganization, muscle protein degradation,[1] cell proliferation,[6, 7] differentiation,[8-10] and vesicular secretion.

Calpastatin, the endogenous inhibitor of calpain-1 and calpain 2, is also ubiquitously expressed, in molar excess compared to the enzymes. Many different splice variants occur in calpastatins, which may lead to different inhibition profiles for the different calpains.[9] It is not clear if calpastatin inhibits calpain 9.

Mutations in calpains have been linked to diseases such as muscular dystrophy and type II diabetes, and calpains also appear to play a role in the caspase system of apoptosis.[11, 12]

Reagent
Anti-Calpain-9 (Domain II, Catalytic Domain), Large Subunit is supplied as approximately 1 mg/ml of antibody in 0.01 M phosphate buffered saline containing 50% glycerol and 0.05% sodium azide.

Storage/Stability
For continuous use, store at 2-8 °C for up to one month. For extended storage, the solution may be stored at 0 °C to –20 °C. Do not store in a frost-free freezer. The antibody is supplied with 50% glycerol to prevent freezing. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

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.

Product Profile
For immunoblotting, a working antibody dilution of 1:1,000 is recommended using an alkaline phosphatase conjugated secondary antibody and a colorimetric substrate such as BCIP/NBT. For chemiluminescent substrates, a working antibody dilution of 1:5,000 is recommended.

For ELISA, immunoprecipitation, and immunohistochemistry, we recommend determining working dilutions by titration.

Note: Higher concentrations of antibody may be needed for samples from more distantly related species. Since calpain 9 is a cellular protein, cell lysates work well for immunoblotting. EDTA/EGTA treatment of tissues or lysates may be required to detect the latent zymogen.

In order to obtain the best results using various techniques and preparations, we recommend determining optimal working dilutions by titration.

References

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