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ProductInformation

Anti-Calpain-10 (Domain T, N-Terminal), Large Subunit
Developed in Rabbit
Affinity Isolated Antibody

Product Number **C 2864**

Product Description

Anti-Calpain-10 (Domain T, N-Terminal), Large Subunit is developed in rabbit using a synthetic peptide corresponding to domain T of the large subunit of human calpain 10 (capn-10) as immunogen. The antibody is affinity purified using agarose to which the immunogen peptide has been bound.

Anti-Calpain-10 (Domain T, N-Terminal), Large Subunit binds to the aminoterminal end of domain T. The antibody detects human, rat, and mouse calpain 10 by immunoblotting. It recognizes the latent and amino-processed protein. The antibody does not crossreact with other calpain family members (calpain 1, calpain 2, calpain 3, etc.). By immunoblotting against the reduced protein, the antibody identifies bands at approximately 75 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 10 is an intracellular cysteine protease. Originally isolated in a search of EST clones, mouse calpain 10 was first named calpain-8,[2] and then renamed to calpain 10 because of the stomach-specific calpain nCL-2 and nCL-3. Calpain 10 was found in all mouse tissues. Calpain 10 polymorphisms have been associated with insulin resistance presumably through decreased mRNA levels.[3] Calpain 10 mutants appear in insulin secretion.[4] Positional cloning studies of type 2 diabetes linked calpain 10 mutants to increased prevalence of type 2 diabetes in Mexican-American families.[5, 6] Since then, many papers have been published on studies of different populations. Some show positive correlation between calpain 10 mutants and type 2 diabetes and others refute the original claim.[7]

Human calpain 10 has been sequenced in ten different isoforms with different carboxyterminal truncations. The predicted proteins range from 672 amino acids (calpain 10A the longest isoform) to 274 amino acids (calpain 10F the shortest isoform). It is unclear whether all of the messages are translated, but PCR of normal human tissues yields a range of bands. The latent large subunit is 75 kDa, and the aminoterminal truncations at activation yield approximately 68 kDa isoforms. Also, a cascade of smaller forms truncated at the N-terminal and C-terminal ends may be seen with further activation.

Unlike the classical calpains (calpain-1 and calpain-2), calpain 10 lacks the EF-hand calcium binding domains, and may not requires calcium for activity, (although ionomycin treatment changes calpain 10 localization[8]). Recombinant calpain 10 expressed in baculovirus[8] is proteolytically active. It is not known if calpain 10 associates with a small subunit as the classical calpains. Calpain 10, 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,[9, 10] differentiation,[11-13] 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.[3] It is not known if calpastatin inhibits calpain 10.

Reagent
Anti-Calpain-10 (Domain T, N-Terminal), 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.

Note: Higher concentrations of antibody may be needed for samples from more distantly related species. Since calpain 10 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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KAA/JPA 07/03