



Product Information

Anti-Calpain-94 (Domain II), Large Subunit

Developed in Rabbit
Affinity Isolated Antibody

Product Number **C 3364**

Product Description

Anti-Calpain-94 (Domain II), Large Subunit is developed in rabbit using a synthetic peptide corresponding to domain II of the large subunit of human calpain 94 (calpain 3, nCANP, nCL-1, calpain p94, muscle calpain) as immunogen. The antibody is affinity purified using peptide agarose to which the immunogen peptide has been bound.

Anti-Calpain-94 (Domain II), Large Subunit detects all isoforms of human calpain 94, including the LP82, 85, 88, and 90 forms. The antibody also detects isoforms 82, 85, 88, and 90 of rat calpain 94. The antibody recognizes latent and active calpain 94. It does not crossreact with other calpain family members (μ -calpain, m-calpain, nCL-2, nCL-3, etc). By immunoblotting against the reduced protein, the antibody identifies bands at 94 kDa, 82 kDa, 62 kDa, 60 kDa, and a series of further cleaved active 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 (m-calpain and μ -calpain), a tissue specific calpain (n-calpain), 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 calpain family members are heterodimers and consist of a common small subunit (regulatory) and a large variable subunit (catalytic). Domains in the large subunit include the aminoterminal domain I, proteinase

domain II, domain III, and EF-hand (Ca^{2+} -binding) domain V.¹

Calpain 94, also known as calpain 3 or muscle calpain, is an intracellular, calcium-dependent cysteine protease. Splice variants of calpain 94 yield a number of different isoforms (LP82, 85, 88, 90), which have a different aminoterminal and different exons spliced or deleted.² The lens contains isoforms LP82 and LP85. The latent large subunit is 94 kDa, and the amino-terminal truncation at activation yields approximately 64-62 kDa isoforms. Also, a cascade of smaller forms can be seen with further activation. Unlike m-calpain and μ -calpain, which are ubiquitously expressed, calpain 94 is a tissue-specific calpain. The half-life of native calpain 94 is also much shorter (approximately 10 minutes) than the other calpains. The endogenous calpain inhibitor, calpastatin, does not inhibit calpain 94. Mutations in calpain 94 have been linked to Limb-Girdle Muscular Dystrophy.³

Calpains are present in all mammalian tissues and are involved in a variety of processes including cytoskeletal reorganization, muscle protein degradation,¹ cell proliferation,^{4,5} differentiation,⁶⁻⁸ and vesicular secretion.

Reagent

Anti-Calpain-94 (Domain II), 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 concentration 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 concentration of 1:5,000 is recommended.

Note: Higher concentrations of antibody may be needed for samples from more distantly related species. Since calpain 94 is a cellular protein, cell lysates work well for immunoblotting. EDTA/EGTA treatment of tissues or lysates is required to detect the latent zymogen.

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

References

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