3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Anti-Calpain-94 (Catalytic Domain), Large Subunit
Developed in Rabbit
Affinity Isolated Antibody

Product Number **C 3239**

Product Description

Anti-Calpain-94 (Catalytic Domain), Large Subunit is developed in rabbit using a synthetic peptide corresponding to the catalytic domain 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 (Catalytic Domain), Large Subunit detects all isoforms of calpain 94. The epitope follows the autocleavage site in insert #1, and thus represents the aminoterminus of cleaved calpain 94. The antibody recognizes latent and active calpain 94 and can be used in various immunochemical techniques including immunoblotting, immunohistochemistry, immunoprecipitation, and ELISA. The antibody does not crossreact with other calpain family members (μ-calpain, m-calpain, nCL-2, nCL-3, etc). It binds to the reduced and non-reduced protein. 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 aminoterminus 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 (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 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.

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 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

1. Johnson, G.V., and Guttmann, R.P., Calpains: intact and active? Bioessays, **19**, 1011-1018 (1997).
2. Nakajima, T., et al., Different expression patterns for ubiquitous calpains and capn3 splice variants in monkey ocular tissues. Biochimica et Biophysica Acta, **1519**, 55-64 (2001).
3. Jia, Z., et al., Mutations in calpain-3 associated with limb girdle muscular dystrophy: analysis by molecular modeling and by mutation in m-calpain. Biophysical Journal, **80**, 2590-2596 (2001).
4. Ariyoshim, H., et al., Possible involvement of m-calpain in vascular smooth muscle cell proliferation. Arterioscher. Thromb. Vasc. Biol., **18**, 493-498 (1998).
5. Kulkarni, S., et al., Calpain mediates integrin-induced signaling at a point upstream of Rho family members. J. Biol. Chem., **274**, 21265-21275 (1999).
6. Balcerzak, D., et al., An antisense oligodeoxyribonecleotide to m-calpain mRNA inhibits myoblast fusion. J. Cell Sci., **108**, 2077-2082 (1995).
7. Murray, S.S., et al., The calpain-calpastatin system and cellular proliferation and differentiation in rodent osteoblastic cells. Exp. Cell Res., **233**, 297-309 (1997).
8. Stockholm, D., et al., Studies on calpain expression during differentiation of rat satellite cells in primary cultures in the presence of heparin or a mimic compound. Exp. Cell Res., **252**, 392-400 (1999).

KAA 08/03