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-μ-CALPAIN
(DOMAIN I), N-TERMINAL
Developed in Rabbit, Affinity Isolated Antibody

Product Number **C 5361**

Product Description

Anti-μ-Calpain (Domain I), N-Terminal, is developed in rabbit using a synthetic peptide corresponding to the amino-terminal of domain I of the large subunit of human μ-calpain (calpain-I, protein kinase C activating factor, E.C. 3.4.22.17) as immunogen. The antibody is affinity purified using agarose to which the immunogen peptide has been bound.

This antibody reacts to the first 30 amino acids of the "propeptide domain," (domain I) of the large subunit of μ-calpain, amino-terminal to the activation cleavage site. It recognizes latent but not active μ-calpain. The antibody can be used to discriminate between the latent and cleaved (active) forms. It binds to μ-calpain, but does not crossreact with other calpain family members (m-calpain, calpain-94, LP-82/85 calpain, nCL-2, nCL-3, etc.). Species reactivity includes human, mouse, and rat. The antibody can be used in various immunochemical techniques including immunoblotting, immunoprecipitation, immunohistochemistry, and ELISA. By immunoblotting against the reduced protein, the antibody reacts with bands at 80 kDa. The antibody also binds the non-reduced protein.

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 amino-terminal domain-I, the proteinase domain-II, domain-III, and EF-hand (Ca^{2+}-binding) domain-IV.[1] μ-calpain is an intracellular, calcium-dependent cysteine protease. It has a micromolar calcium sensitivity (thus the μ-) as compared to the millimolar calcium sensitivity of m-calpain. Both μ-calpain and m-calpain are ubiquitously expressed, and are countered by the endogenous calpain inhibitor, calpastatin. μ-calpains consist of a latent large subunit (80 kDa) that is activated by amino-terminal truncation to yield an approximately 58 kDa form. Cleavage of the carboxyl-terminal region generates smaller forms of μ-calpain, but it is not clear if these forms are proteolytically active.

Most cell types produce μ-calpain. Calpains are present in all mammalian tissues and are involved in a variety of processes including cytoskeletal reorganization, muscle protein degradation,[1] cell proliferation,[2, 3] differentiation,[4-6] and vesicular secretion.

Reagent

Anti-μ-Calpain (Domain I), N-Terminal is supplied as 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 antibody is recommended.

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

Note: Higher concentrations of antibody may be needed for samples from more distantly related species. Since μ-calpain is a cellular protein, cell lysates work well for immunoblotting.

In order to obtain the best results using different techniques and preparations we recommend determining the optimal working concentrations by titration.

References

1. Johnson, G.V., and Guttmann, R.P., Calpains: intact and active? Bioessays, **19**, 1011-1018 (1997).
2. Ariyoshi, H., et al., Possible involvement of m-calpain in vascular smooth muscle cell proliferation. Arterioscler. Thromb. Vasc. Biol., **18**, 493-498 (1998).
3. Kulkarni, S, et al., Calpain mediates integrin-induced signaling at a point upstream of Rho family members. J. Biol. Chem., **274**, 21265-21275 (1999).
4. Balcerzak, D., et al., An antisense oligodeoxyribonucleotide to m-calpain mRNA inhibits myoblast fusion. J. Cell Sci., **108**, 2077-2082 (1995).
5. 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).
6. 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 11/01