

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-13 (Domain III, N-Terminal), Large Subunit Developed in Rabbit Affinity Isolated Antibody

Product Number C 3114

Product Description

Anti-Calpain-13 (Domain III, N-Terminal), Large Subunit is developed in rabbit using a synthetic peptide corresponding to domain III of the large subunit of human calpain 13 (capn-13) as immunogen. The antibody is affinity purified using agarose to which the immunogen peptide has been bound.

Anti-Calpain-13 (Domain III, N-Terminal), Large Subunit recognizes domain III in the large subunit of human calpain 13 by various immunochemical techniques including immunoblotting, immunoprecipitation, immunohistochemistry, and ELISA. It recognizes both the latent and active protein. The antibody detects human, rat, and mouse calpain 13 and does not crossreact with other calpain family members (calpain 1, calpain 2, calpain 3, etc.). The antibody binds to the reduced and non-reduced protein. By immunoblotting against the reduced protein, the antibody identifies bands at approximately 64 kDa, 52 kDa, 48 kDa, 40 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 (calciumdependent 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 (Ca²⁺-binding) domain IV.¹

Calpain 13, also known as capn-13 and muscle calpain, is an intracellular cysteine protease. The sequences for calpain 13 and calpain 14 were discovered in a search of human ESTs.² Both genes are located on chromosome 2p22.2-22.3 separated by 447 kb (calpain 13 is 65 kb and calpain 14 is 26 kb in length). Three different calpain 13 sequences have been discovered thus far, differing in the last 120 amino acids. The original published sequence codes for a protein of 557 amino acids, predicting a molecular weight of 63.6 kDa. A sequence derived from the NCBI annotation project begins at 443 amino acids into the original sequence, and codes for 223 amino acids. There are a few differences in the overlapping sequences, but an assembled sequence codes for a protein of 669 amino acids with a molecular weight of 76.7 kDa. Unlike the classical calpains (calpain 1 and calpain 2), calpain 13 does not have the EF-hand calcium-binding domain IV. Instead, calpain 13 (similar to calpains 5, 6, 7, and 10) has a modified domain III, called domain T. It is not known if calpain 13 is calcium dependant or which potential isoforms of calpain 13 are produced in vivo.

Calpains are present in all mammalian tissues. The calpain 13 sequence has been detected in lung and testis and may have wider distribution. Calpains are involved in a variety of processes including cytoskeletal reorganization, muscle protein degradation,¹ cell proliferation,^{3, 4} differentiation,⁵⁻⁷ and vesicular secretion.

Reagent

Anti-Calpain-13 (Domain III, 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.

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 13 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

- Johnson, G.V., and Guttmann, R.P., Calpains: intact and active? Bioessays, 19, 1011-1018 (1997).
- 2. Dear, T.N., and Boehm, T., Identification and characterization of two novel calpain large subunit genes. Gene, **274**, 245-252 (2001).
- Ariyoshim, H., et al., Possible involvement of m-calpain in vascular smooth muscle cell proliferation. Arterioscher. Thromb. Vasc. Biol., 18, 493-498 (1998).
- Kulkarni, S., et al., Calpain mediates integrininduced signaling at a point upstream of Rho family members. J. Biol. Chem., **274**, 21265-21275 (1999).
- Balcerzak, D., et al., An antisense oligodeoxyribonecleotide to m-calpain mRNA inhibits myoblast fusion. J. Cell Sci., **108**, 2077-2082 (1995).
- 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).
- 7. 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).

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